Studies on Fusarium wilt of oil palm

Mepsted, Roger Charles

Award date:
1992

Awarding institution:
University of Bath

Studies on *Fusarium* Wilt of Oil Palm

submitted by

Roger Charles Mepsted

for the degree of PhD of the University of Bath

1992

signature

ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106-1346

Man *dwells* naturally within the tropics and lives on the fruit of the palm tree. He *exists* in the other parts of the world and there makes shift to feed on corn and flesh.

Linnaeus

ABSTRACT

In West Africa the most serious disease of oil palm (*Elaeis guineensis*) is vascular wilt caused by *Fusarium oxysporum* f. sp. *elaeidis*. This is the first investigation of the physiology and growth of diseased plants. Leaves on infected plants suffer from varying degrees of water stress. Leaf stunting, a prominent symptom, was associated with prolonged mild water stress and a relatively small increase in vascular resistivity in the petiole, while nonstunted leaves, formed before the pathogen became established, were severely water stressed and had very high vascular resistivity in the petioles.

Stunting was primarily due to a reduction in cell division and could be simulated by the application of an inhibitor of gibberellin synthesis, and partly reversed by the application of GA3. Stunting was also associated with a significant reduction in xylem diameter, which in combination with the reduced leaf area may represent an adaptation to disease induced water stress.

Host resistance is the only practical control method for this disease. This study has resulted in significant improvements to existing nursery testing procedures and a dependable method for the screening of clonal material. This technique facilitates study of host resistance and variation in the aggressiveness and virulence of pathogen isolates.

A method using an increment borer was developed which, for the first time, permitted the non-destructive quantification of internal infection in mature palms. Analysis of core samples from the trunk revealed that in some crosses many symptomless palms were infected. In diseased palms, vascular blockage was correlated with external symptoms and was only found in the stem and not in the roots. Applications for the selection of disease resistant palms in the field are discussed.

Preinoculation of young palms with an invasive but non-pathogenic Malaysian soil isolate of *F. oxysporum* produced varying degrees of disease control. It is possible that exclusion of pathogenic isolates from Malaysia is due to the activities of such strains of *F. oxysporum*. However, outbreaks of this disease in South America, probably resulted from introduction of the pathogen on contaminated seed from Africa. Therefore, methods for the decontamination of palm material were required, and this was achieved by two novel techniques, vacuum infiltration of fungicide into seed, and the application of fungicide in organic solvents to pollen.

The pathogen has previously been regarded as soil- or seed-borne but soil isolates of *F. oxysporum* from an infected plantation were non-pathogenic. However, isolates were found in high numbers on old male inflorescences and in the air; all of these isolates were pathogenic. These observations have important implications for the epidemiology of this disease.

Acknowledgements

These investigations were sponsored by Unilever Plantations Group. I would like to thank Cyril Nyanduza, Hans and Madelon Van Amstell at the Joint Research Scheme at Binga in Zaire, and Roland Porter, Hereward Corley and Gail Smith at Unilever Plantations Group in Bedford for all their help and cooperation. In particular, thanks are due to Drs Corley and Smith for their helpful and critical comments on chapter three.

At Bath University I would like to thank all my friends and colleagues for all their encouragement; particular thanks are due to Mike Skinner and Ray Dixon for their technical support.

Finally, I am very grateful to my supervisors Julie Flood and Richard Cooper for their endless patience, encouragement and guidence throughout the duration of this thesis.

CONTENTS

List of Abbreviations

Foe *Fusarium oxysporum f. sp. elaeidis*

IRGA Infra red gas analyser

PBZ Paclobutrazol

PFD Photo flux density

RH Relative humidity

RTV Resistivity

RWC Relative water content

1.0. GENERAL INTRODUCTION

Oil Palm (*Elaeis guineensis* Jacq.) is a large monoecious feather palm with a columnar stem and short internodes (Hartley, 1988). It is now grown throughout the tropical regions of the world but is thought to have originated from the forests of W. Africa. Here it occurs most frequently as semi-wild groves, in areas where forest has been cleared.

It has been exploited by man for many centuries for the production of palm wine and oil; the latter is extracted from the fruit (both kernel and pulp) which are borne on large spiky bunches that develop in the leaf axils.

Although small quantities of palm oil have been imported into Europe from W. Africa since at least the 16th century, it was only with the abolition of slavery and the industrial revolution that a significant increase in trade occurred. The oil was used for the production of soaps, candles, margarines and as a lubricant and was supplied from native exploitation of semi-wild groves. Early in this century the increase in demand, stimulated the construction of commercial mills and the establishment of plantations in the Far East and Africa. Since then the oil palm has become established as a plantation crop throughout the tropics, particularly in the Far East, which now supplies the majority of world demand for palm oil (Hartley, 1988).

Although world-wide oil palm may be regarded as a commercial plantation crop, in many parts of Africa this is not the case. For example, in Nigeria (where palm oil is considered to be an important part of the national diet) < 20% of oil production is from commercial estates, while > 60% comes from exploitation of semi-wild groves (Anon, 1991). Such native exploitation is constrained by poor agronomic practices and the low yield potential of semi-wild palms (Hartley, 1988). However, high yielding tenera seed varieties have been developed and are now available to small-holders in several African countries (Hartley, 1988; Anon, 1991; Kullaya, 1991)

There is no natural form of vegetative propagation in oil palm and thus it has not been possible in the past to multiply selected palms to produce high yielding clones. However, with the development of tissue culture techniques for oil palm, this problem has been overcome and will allow the production of good quality clonal material with significant yield improvements (Jones, 1983).

In the past 30 years, whilst production in the Far East has increased, exports from W. Africa have declined due to civil disorder and increased internal demand (Purseglove, 1985; Hartley, 1988). In addition, production in Africa has also been constrained by *Fusarium* wilt which is regarded as the most serious disease of oil palm in Africa (Renard, 1976; Turner, 1981).

Fusarium wilt of oil palm is a vascular wilt disease

and is caused by the fungus *Fusarium oxysporum* Schl. f.sp. *elaeidis* Toovey (Foe). In general vascular diseases are highly destructive, and can be caused by fungal or bacterial pathogens. Wilt pathogens enter the vascular system of the host and remain in the conductive xylem tissue until the infection is well advanced (Green, 1981). The majority of fungal wilt diseases are caused by species of *Fusarium* or *Verticillium*. Generally, infection leads to symptoms of water stress such as wilting, also chlorosis and necrosis of leaves; vascular browning is usually found and xylem vessels become occluded with tyloses, gums and gels. Symptom development in this group of diseases and its possible causes are discussed further in Chapter 3.

Fusarium oxysporum has a world-wide distribution and is a common saprophyte, although some strains are plant pathogens. Some pathogenic forms are poorly specialised and cause seedling blights, necrosis or rots. However, the forms responsible for vascular wilts exhibit a high degree of host specificity, on the basis of which they have been divided into >80 *formae speciales* (ff. spp.) (Louvet, 1988). Such ff. spp. have been further divided into races where they exhibit selective pathogenicity within a host species.

Vascular wilt of oil palm was probably first recorded in Nigeria in 1944, but was originally thought to be a physiological disorder (West, 1944; cited by Turner, 1981). The pathogen was identified in Zaire 2 years later

(Wardlaw, 1946a,b,c) and was confirmed in Nigeria (Wardlaw, 1948), Cameroon (Anon, 1960), Ivory Coast and Dahomey (Renard, Gascon & Bachy, 1972). There have also been unconfirmed reports of the disease from S. America, in Surinam (Anon, 1951) and Colombia (Sanchez Potes, 1966). While more recently, the pathogen has been isolated and identified in Brazil (Van de Lande, 1983) and Ecuador (Renard & de Franqueville, 1989). The disease has never been reported in the Far East or Papua New Guinea. The world-wide distribution and epidemiology of this disease is discussed more fully in Chapter 5.

The pathogen can attack oil palm at any age, from seedling to mature palm, and Prendergast (1957) suggested that in mature palms the disease could exist in 2 forms. In the more usual chronic form, the older leaves become desiccated and the rachis breaks near or at some distance from the base. The disease proceeds gradually, with younger leaves becoming successively affected, while the erect young leaves in the crown are much reduced in size and may become chlorotic; the palm can exist in this condition for several years. Less frequently, a palm may display the acute form of the disease, in which leaves dry out and die rapidly whilst still in an erect position before snapping off, usually at some distance from the base. The disease progresses rapidly and palms die within 2 or 3 months. Intermediate stages between these extremes are also encountered. Recently, de Franqueville & Renard (1990) have suggested a third category of temporary wilt,

where palms develop leaf symptoms but later recover. At the nursery stage, infected plants show progressive shortening of younger leaves followed by desiccation and death of older leaves (Prendergast, 1957).

Internally, this disease is characterised by vascular discoloration and blockage of xylem vessels with tyloses and gums (Kovachich, 1948; Ho, Vargese & Taylor, 1985b). Vascular discoloration is most often observed in the stem of palms, although in severely infected plants it can also occur in the petiole (Turner, 1981). However, even in highly diseased field palms most roots show no signs of infection (Wardlaw, 1950; Prendergast, 1957).

Infection is thought to occur through the roots, and Renard (1970) stated that the fungus was unable to penetrate undamaged roots, but Fraselle (1951) and Locke & Colhoun (1977) showed that infection could occur through roots that had not been deliberately damaged. However, in these experiments inoculation occurred at the same time as palms were transplanted, so some degree of root damage was probable. More recently, Flood, Cooper & Lees (1989) demonstrated that it was possible to infect seedlings whose roots had not been damaged or disturbed. Locke & Colhoun (1977) observed the fungus penetrating the loosely packed cells at the base of the pneumathodes (short modified roots in which the exodermis has ruptured, so exposing the cortical tissue and stele) and suggested that this was the prime entry site.

Vascular wilt has been reported as a minor disease of palms in semi-wild groves (Waterson, 1953; Oritsejafor, 1989), while losses of 20 to 50% of palms under 10 years old have been reported in some plantations (Wardlaw, 1950; Waterson, 1953; Guldentops, 1962; Renard & Quillec, 1983). However, generally losses are less severe, and have been estimated at between 1 and 2 % per annum (Bachy, 1970; de Franqueville & Renard, 1990), and in some area of W. Africa wilt has never been observed in groves or plantations (Waterson, 1953; Aberungboye, 1981). Indeed, Hartley (1988) indicated that the early menacing reputation of this disease may have been over estimated, since no major epidemic had occurred in W. Africa.

Prendergast (1957) suggested that no yield reduction could be expected until > 20% of palms had died, due to the increased vigour of adjacent palms. However, he warned that this yield adjustment could only be expected if diseased palms died quickly, and that infected yet tolerant palms may cause an overall reduction in yield. The influence of tolerant plants on yield was demonstrated by Renard & de Franqueville (1989) who reported a 6 to 16% yield reduction in 6 year old palms where the incidence of plants with obvious symptoms of wilt was only 2.5 to 5.5%. They concluded that most of the yield reduction was caused by the 20 to 30% of apparently healthy palms which were infected yet had less obvious symptoms.

Environmental factors have also been reported as affecting the incidence of wilt. Prendergast (1957) and

Aberungboye (1981) observed higher levels of disease in areas of low rainfall, and Prendergast (1957) suggested that drought-induced root death made palms more susceptible to wilt. Waterson (1953) also observed that most new cases of wilt occurred at the end of the rainy season.

In addition, cultural practices can influence disease incidence. Higher levels of wilt have been reported when oil palm has been replanted on sites of previously infected palms (Prendergast, 1957; Renard & Quillec, 1983). However, such replanting cannot always be avoided since the crop must be located within a reasonable distance of the processing factory (Van Amstel, 1990, pers comm). The application of potassium (Prendergast, 1957; Renard & Quillec, 1983; Renard & de Franqueville, 1989) and removal of ground cover plants (Renard & Quillec, 1983) reduced the incidence of wilt, while the application of spent bunch stalks, after factory processing (therefore presumably sterile) to palm bases increased disease levels (Renard & de Franqueville, 1989).

Although wilt has been controlled at the nursery stage by the application of a fungicide, this technique was regarded as uneconomical on the scale of a plantation (Renard, 1976). Therefore the majority of research into the control of this disease has been concentrated on the development of resistant or tolerant varieties. This topic and possible improvements to screening for disease resistance are discussed more fully in Chapter 4.

Although *Fusarium* wilt has been studied for > 40 yrs there are still several important aspects of this disease that have either never been investigated or remain unclear. In part this reflects the difficulty of working on a tropical crop with a long breeding cycle, but it is also due to the sometimes variable, and always slow, development of symptoms in inoculated seedlings and naturally infected field palms. Thus, the mechanisms involved in disease resistance have (until recently) been ignored, and the physiology and likely cause(s) of yield reduction of diseased palms has never been studied. Furthermore, investigations of epidemiology have been largely confined to the distribution and frequency of diseased palms rather than the spread of the pathogen.

The main aims of this work are therefore:-

1. To investigate the water relations and growth of diseased palm.

2. To improve the resistance screening procedures at the nursery and field trial stage.

3. To investigate the population level of Foe in the plantation, and the spread of the pathogen on seed and pollen.

2.0.MATERIALS AND METHODS

2.1.Plant Material and Growing Conditions.

Oil palm seeds (from Plantation Lever au Zaire, Binga, Zaire) were supplied either heat treated or requiring heat treatment to initiate germination (Hartley, 1988). Seeds requiring heat treatment were soaked in water for 7 days at 25°C with daily changes of water. They were then air dried to a dull black colour, sealed in heavy duty plastic bags and heat treated by incubating at 39°C for 80 days. These seeds and those already heat treated in Zaire were then soaked in water at 25°C for 7 days and air dried as above, sealed in plastic bags with an equal volume of air and incubated at 26° to 27°C. Germination started 2 to 7 days after incubation and usually reached a maximum after 2 weeks.

Seedlings were transplanted to seed trays (300x220x50 mm) filled with compost (Levingtons F2, Levingtons M2, Perlite, in ratio 1:1:1) and maintained in a controlled environment cabinet (28°C 80% RH and 12 hour day with a light level of 240 μ mol $m^{-2}sec^{-1}$ photo flux density [PFD]). At the first leaf stage, they were transplanted into black polyethylene pots (Plastics by Post, Isle of White) (80x190 mm containing 1.2L of compost) and transferred to the glasshouse.

Clonal plantlets, (supplied by Unifield T.C. Ltd, Bedford.) were initially transferred from test tubes to the seed trays described previously. To protect these delicate plantlets, propagator lids were placed over the

trays to maintain a RH of 100%. The RH was reduced, over 2 months, to about 80% when the palms were transplanted to the polyetheylene pots and transferred to the glasshouse.

To prevent splash-borne contamination between treatments, 7 or 8 pots of the same treatment were placed in plastic troughs (610 x 160 x 140 mm, B-Line, Swansea). By the fourth leaf stage, the number of plants per trough was reduced to 3 or 4 to prevent overcrowding. Each month, plants within each treatment were randomised between the troughs for that treatment and the troughs were rerandomised in the glasshouse. To further reduce splash contamination plants were watered from below, as required. Once a month, palms were watered with a liquid fertiliser (Fisons Liquinure, 1 in 45 dilution, containing N, P, K. in the ratio 8:4:4 and trace elements), Soil pH ranged from 5.0 at the start of the experiment to 6.4 after 5 months.

Conditions in the glasshouse varied according to the season, but by careful use of shading and artificial lights (Camplex 500 W metal halide), light levels were maintained between 800 and 500 μ mol $^{m-2}$ Sec^{-1} with a day length of 14 to 17 h; humidity and temperature ranged from 60 to 90% and 22 to 35^{o}C respectively.

2.2. INOCULUM PRODUCTION AND STANDARD INOCULATION PROCEDURE.

A single spore isolate (F3) of *F. oxysporum* f. sp. *elaeidis* (Foe, identity confirmed by I.M.I.) from a diseased palm in a field microplot at Binga in Zaire was used in all experiments. The pathogenicity of this isolate has been compared with others from Zaire (Flood, 1987, pers comm) and is believed to be a representative African isolate of Foe (Flood *et al*, 1992). Stock cultures were stored on sterile soil at 7°C to minimise spontaneous mutation. In order to produce inoculum a small quantity of soil was plated onto potato dextrose agar (Oxoid, Basingstoke),(Appendix 1) and incubated for 3 to 5 days at 28°C, then five discs (5mm diameter) were removed from the actively growing edge of the cultures and placed in a 250ml conical flask containing 100ml of sucrose salts medium (Cooper & Wood, 1975),(Appendix 1). The fungus was cultured for 3 days in an orbital incubator at 100 rpm and 28°C. The fungal suspension was then filtered through 2 layers of muslin , to remove mycelial fragments, and the concentration of microconidia calculated with a haemocytometer. The spore concentration was adjusted to $3.3x10^7$ ("high") or $3.3x10^6$ ("low") spores ml^{-1} with sterile distilled water.

Ten ml of spore suspension was applied, with a syringe, onto the soil surface around the base of each palm. To simulate the effect of nutrients on soil microflora, uninoculated plants (controls) received 10ml

of 10% sucrose salts medium. Unless otherwise stated, clonal plants were inoculated 2 to 3 weeks after transplanting, whilst seedling palms were inoculated at the 2 leaf stage.

2.3. Assessment of Plant Growth and Disease Development

Unless otherwise stated, the methods described below refer to measurements of young seedling or clonal palms.

2.3.1. Plant Growth and Disease Development

2.3.1.a Plant Height.

Plant height measurements were taken from soil level to the tip of the longest leaf. However, due to the position of the growing point in palms, plant height does not increase until the youngest expanding leaf has grown above the height of the previous leaf. Thus, although this assessment was subsequently improved by measuring the height of the youngest fully expanded leaf, it is not an ideal indicator of overall plant growth. Consequently more detailed, non-destructive, measurements of growth were obtained by estimating the lamina area of each leaf.

2.3.1.b. Leaf Area.

From the time of inoculation, leaves were numbered as they developed using a marker pen on the petiole. Leaf 1 was the youngest fully expanded leaf at the time of inoculation and successive leaves were numbered as they developed. Therefore, the larger the leaf number the younger the leaf.

The area of each leaf lamina was calculated from measurement of lamina length and maximum width. Thus leaf area (mm^2) = length (mm) x width (mm) x calibration factor. The calibration factor was calculated by plotting the product of leaf width x length against the actual leaf area, which was calculated by weighing pieces of paper (of known weight per unit area) cut to the shape of the leaf. For pinnate leaves, lamina length was estimated by the addition of leaflet widths.

Correlation and regression analysis for these data were performed for randomly selected leaves from healthy and diseased palms of 3 seedling crosses and clone UF4. Despite the change in leaf shape (from lanceolate to bifurcate to pinnate) that occurs as young palms develop the correlation coefficient of leaf measurement against area was always highly significant ($p < 0.001$). There was no variation in calibration factor between stunted leaves (from diseased palms) and those from uninfected palms. However, there was some variation in calibration factor between the different crosses and clones, but due to the linear nature of the calculation involved and the small variation in calibration factor (0.565 - 0.609) a mean value of 0.587 was adopted for all calculations of leaf area.

Leaf area measurements of field palms were made at Binga, Zaire by Dr Gail Smith (Unilever Plantations Group) and staff of the Joint Research Scheme (Plantation Lever au Zaire and Societe du Culture au Zaire) using a standard

formula (Hardon, Williams & Watson, 1969). Ten leaflets were sampled from each side of the rachis, three fifths of the way up the leaf; the width and length of the six longest leaves were then measured and the data entered into the following formula:-

Leaf area (m^2) = (length x width, leaflet 1 +..... length x width, leaflet 6) x total number of leaflets x $9.167x10^{-6}$

2.3.1.c. Leaf Production

Leaf production was assessed by counting the number of fully expanded leaves and estimating the development of any partly expanded leaves. The latter was obtained by dividing the current (partly expanded) leaf area by the fully developed leaf area, obtained from later recordings.

2.3.1.d. Leaf Cell Size and Air Space Volume.

Leaf cell size was determined from microscopic measurement of sections of leaf lamina and petiole (previously fixed in a solution of formalin, acetic acid, ethyl alcohol and water, 13:5:100:100),

The surface area of upper epidermal cells in the leaf lamina were calculated from length x width measurement of a line of cells, in the middle of the leaf at right angles to the petiole. The volume of spongy mesophyll cells was estimated from measurements of cell diameter in longitudinal and transverse sections, with the presumption that the cells were perfect spheres. The volume of petiole parenchyma cells was calculated from length and diameter

measurements based upon the premise that these cells were elongated cylinders.

The volume of internal air spaces in the leaf lamina was estimated by the method of Cosgrove & Cleland (1983); 11mm leaf discs were weighed , infiltrated with silicone oil (Fluid 200, 1 centistoke viscosity, Dow Corning.) under partial vacuum (until air bubbles no longer emerged from the tissue), wiped dry and reweighed. The percentage air volume (to weight) was calculated from the increase in weight and the density of the oil.

2.3.1.e. Dry Weight.

Final growth measurements were obtained by dry weight analysis, following 48 h at 80^{o}C in a drying oven.

2.3.2. Foliar and Internal Symptoms.

2.3.2.a. Leaf Chlorosis and Necrosis.

At intervals, the percentage chlorotic or necrotic leaf area was estimated using 2 alternative methods. By the first method, each leaf was examined and the percentage affected area estimated. In the second method, a chlorosis and necrosis index was used with plants being rated from 0 to 5, (0=healthy, 1=slight chlorosis of oldest leaves, 2=significant chlorosis of < 20% of leaves, 3=significant chlorosis of < 40% of leaves, 4=significant chlorosis of > 40% of leaves, 5=plant dead.).

2.3.2.b. Browning of Stem Tissue in the Bulb of Young Palms.

Plants were split longitudinally through the swollen stem base ("bulb") and the percentage area of the bulb stem tissue with browning was assessed using a stem area key (Fig 1a). The bulb stem tissue was defined as the central part of the bulb and excluded leaf bases (Fig 1b).

2.3.2.c Browning of Vascular Tissue in Field Palms.

Two, 400mm, tree increment borers (Mattesson, Sweden) were used to remove cores (5.15mm diameter) from the trunk of diseased and healthy palms in Zaire. The borers consist of a threaded hollow tube that was screwed into the trunk to the required depth (Fig 2a), and a trunk core was then removed with a hooked extractor (Fig 2b). Cores were cut longitudinally, at right angles to vascular bundles, and examined with a hand lens (Fig 2c) and a microscope (Fig 2d); any brown vessels were noted and the percentage of vessels so affected was calculated. Distinction was made between browning of xylem vessels elements induced by *Fusarium* wilt and that of vascular bundle fibres which could be due to *Ganoderma* infection (Wardlaw, 1950).

The level of browning and occlusion of root xylem vessels was estimated by examining samples of primary root taken from the base of field palms; the percentage infected vessels was calculated as above.

Figure 1a: Stem Tissue Area Key for Assesment of Stem Browning in Young Palms

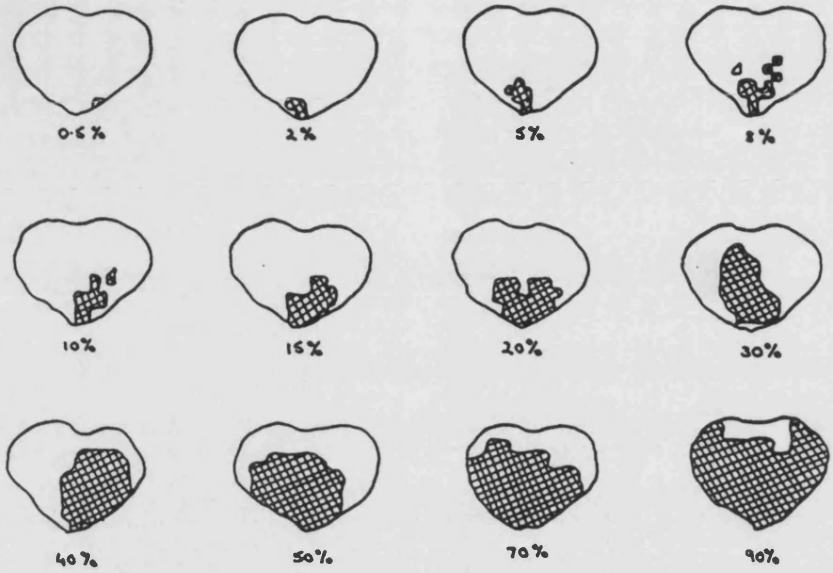


Figure 1b: Longitudinal Section throught the Bulb of an Infected Seedling, Brown Stem Tissue Indicates Infection.

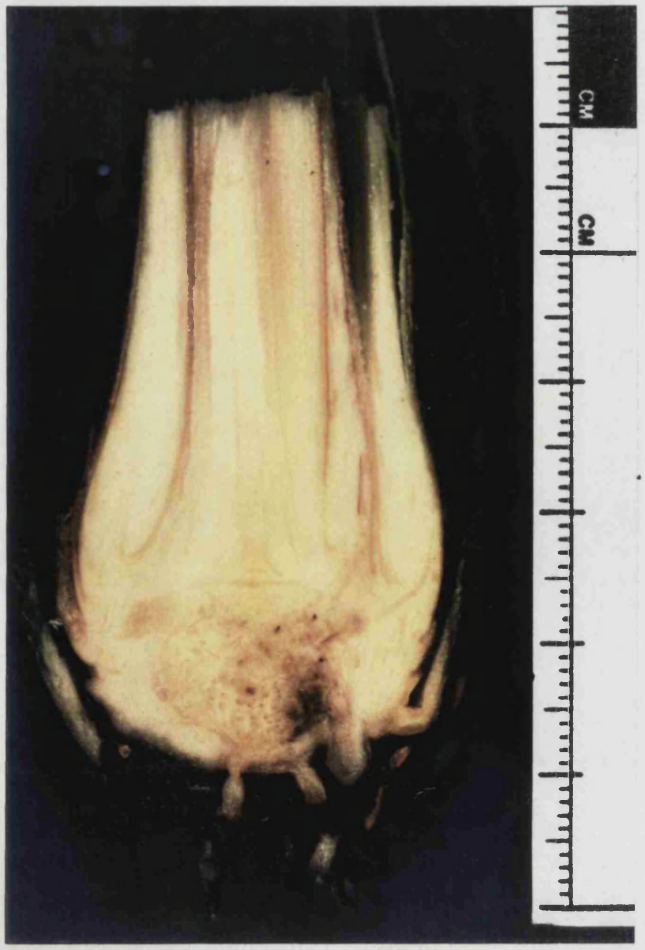
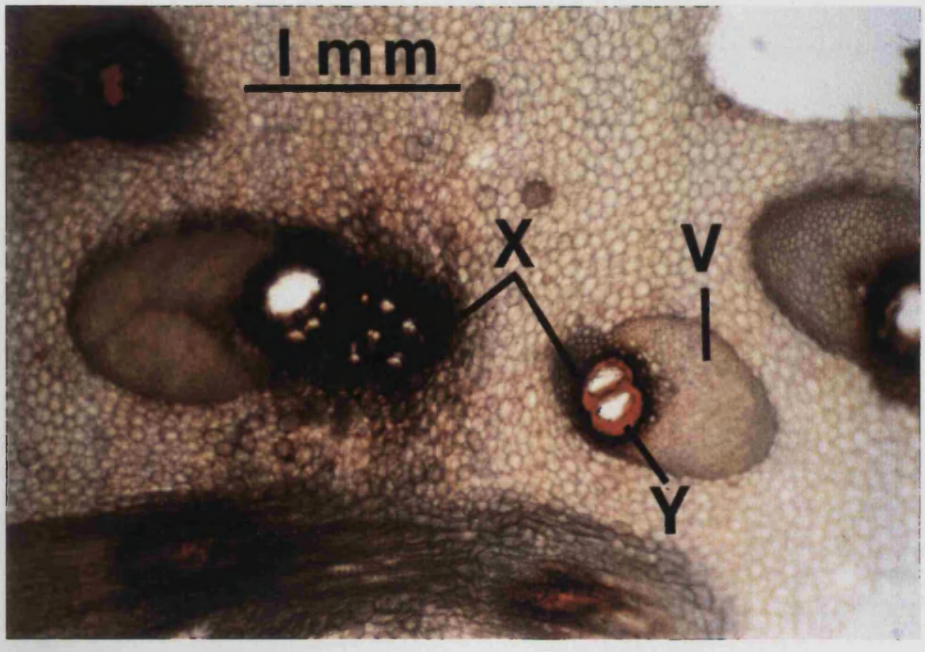


Figure 2a: Insertion of Incement Borer into a Palm Trunk.

Figure 2b: Removal of a Trunk Core from the End of the Borer; Dark Spots in the Core Indicate Vascular Browning.

Figure 2c: Longitudinal Section of a Trunk Core, at Right Angles to Vascular Bundles, from (A) an Infected Palm with Brown Vascular Bundles (Arrowed) and from (B) an Uninfected Palm.

Figure 2d: Micrograph of Trunk Tissue Showing Browning of Xylem Parenchyma (X) and Gel Deposition Within Xylem Vessels (Y); Note the Absence of Browning of Vascular Fibres (V).



2.3.3. Reisolation and Quantification of the Pathogen from Plant Material

For qualitative reisolation, fragments of plant material (1 cm sections of petiole or stem core sample) were surface sterilised in sodium hypochlorite (10% v/v, 1% available chlorine) for 10 mins (5 mins for auger cores) before rinsing twice in sterile distilled water. The material was then plated onto *Fusarium* selective medium (Papavizas, 1967) augmented with antibiotics (Appendix 1) and incubated for 4 days at 28^{o}C under near U.V. light.

For quantitative reisolation, *ca.* 0.5g of stem tissue from the centre of the bulb was surface sterilised and washed as above. The tissue was ground with a pestle and mortar with 1cm^{3} of acid washed sand and 9ml of sterile distilled water. A ten fold dilution series was prepared and plated onto duplicate plates of *Fusarium* selective medium. After incubation, as described above, colonies of *F.oxysporum* were counted and the number of colony forming units (cfus) per g fresh weight of palm tissue was calculated.

2.3.4. Plant Transpiration and Photosynthesis.

In all cases measurements of transpiration and photosynthesis where conducted under conditions of high light intensity (> 500 μmol m^{-2} s^{-1} photon flux density) in which they were considered to be light saturated (Smith, 1989).

2.3.4.a. Measurements of Transpiration by Weight Loss

Total water loss from individual palms,over a set period, was calculated by the difference in weight of plant plus pot minus the mean weight loss of pots without palms. In several experiments, these data were combined with leaf area measurements to produce estimates of water loss per unit leaf area.

2.3.4.b. Porometer Measurements of Stomatal Resistance.

An automatic porometer, Mk2 Delta-T Devices (Delta T-Devices, Cambridge) was used for the measurement of abaxial stomatal resistance by water vapour diffusion. The porometer consists of a humidity sensor that is clamped to the leaf surface. Leaf transpiration is then allowed to humidify a narrow recess (the cup) in the sensor head. The increase in humidity in the cup is timed over a fixed interval of RH, and the time taken for humidity to increase is related to the stomatal resistance of the leaf. The porometer was used and calibrated as described by the manufacturer, but it was found necessary to use the machine for about 20 mins on nonexperimental plants to obtain stable readings. The porometer was attatched half way along the lenght of the leaf, to one side of the midrib and between major viens.

2.3.4.c. Infra Red Gas Analyser Measurements of Stomatal Conductance and Photosynthesis.

Both photosynthetic rate and stomatal conductance (inverse of stomatal resistance) were calculated using an Analytical Developments LCA2 portable I.R.G.A. (Analytical Developments, Hoddesdon). In this system, part of the leaf

was enclosed in a chamber in which air temperature, humidity, light intensity and atmospheric CO_2 were monitored. Increases in humidity and decreases in CO_2 concentration of air passing through the chamber were automatically combined with the air flow rate and energy flux measurements to obtain values of stomatal conductance and photosynthesis; from these data an estimate of substomatal CO_2 was also calculated.

2.3.5. Measurements of Leaf Water Potential

A Wescor HR 33T Dew Point Microvoltmeter (Wescor Inc, Utah, U.S.A.) was used to estimate the water potential of leaf discs by the methods recommended by the manufacturer. Both the psychrometer and dewpoint (hygrometer) modes of operation were used but it was found impossible to obtain accurate readings, therefore this method was abandoned in favour of using a pressure chamber.

A standard Cook water potential apparatus (Chas. W. Cook & Son, Birmingham) was operated following the principles of Scholander, Hammel, Bradstreet & Hemmingsen (1965). The apparatus consisted of a pressure vessel into which an excised leaf was inserted with only the cut end of the petiole protruding. Pneumatic pressure was applied to the chamber from a cylinder of nitrogen gas, the pressure in the chamber was slowly increased (< 0.5 MPa per minute) until fluid was observed at the cut petiole surface.

This pressure (the balance pressure) was read from a pressure gauge and is considered to be equal and opposite

to the leaf mesophyll water potential (Boyer, 1967; Passioura, 1991). This pressure is not equivalent to xylem water potential, because when the petiole is cut the tension in the xylem is released and the xylem sap equilibriates with the water potential of the leaf. However, xylem water potential can be expected to be very similar to leaf water potential and, due to the very low osmotic potential of sap, the leaf water potential measured with this apparatus provides a good estimate of xylem water potential (Passioura, 1991).

2.3.6. Calculation of Leaf Relative Water Content.

The fully turgid weight and relative water content (RWC) of leaves was obtained by the method of Ladiges (1975). Thus, a leaf was weighed before placing in the pressure chamber , which was pressurised to estimate leaf water potential (as described above). Then, in stages up to ca. 0.7 MPa (100 p.s.i.). Fluid, forced from the petiole, was collected onto lens tissue packed into a 1ml disposable pipette tip which was mounted over the cut end of the petiole. At each pressure, the quantity of exudate was calculated by weighing the pipette and lens tissue, it was then possible to determine the fresh weight of the leaf at each pressure (fresh weight when placed in pressure chamber minus the weight of exudate). By plotting these values of weight against pressure, it was possible (by back projection to pressure = 0) to estimate the weight of the leaf at zero xylem pressure (potential fully turgid weight). Leaves were then air dried (80^{o}C for 24h)

and the RWC of the leaf when placed in the pressure chamber was calculated.

2.3.7. Calculation of Leaf Solute Potential.

Initially, estimates of leaf solute potential were made following the methods of Scholander *et al* (1965), Tyree & Hammel (1972) and Ladiges (1975). Thus, the procedure described above for estimating RWC was followed except pressure was applied in stages up to ca. 2.5 MPa. Data of inverse balancing pressure was then plotted against RWC at that pressure. By back and forward projection of the linear part of this curve, leaf solute potential and cell wall water content could be respectively estimated. Unfortunatly, this procedure was very time consuming and the variable and high estimates of cell wall water content so obtained (15-50 % of total water) indicated that accurate measurements were not always obtained.

An alternative method was therefore developed based upon techniques for the collection of apoplast fluid following over-pressurisation in a pressure chamber (Cosgrove & Cleland, 1983; Hartung, Rabin & Hendrix, 1988; Meinzer & Moore, 1988).

Apoplast fluid was collected by pressurising the chamber to 1.4 MPa; this pressure was chosen because graphs of inverse balance pressure against RWC (as above) indicated that zero cell turgor would occur at higher pressures and consequently, apoplast fluid would be

diluted by a comparatively large volume of symplastic water. Exudate at the cut petiole surface was collected on lens tissue packed inside a 1ml plastic syringe placed over the petiole. Fluid was later squeezed from the syringe, by inserting the plunger, and stored in plastic Eppendorf tubes in liquid nitrogen for between 1 to 3 h before determination of osmolality.

To collect symplast fluid, the leaf was removed from the pressure chamber, frozen over liquid nitrogen, thawed, replaced in the pressure chamber and pressurised to 2.0 MPa; the exudate was collected and stored as described above. Leaves were frozen over liquid nitrogen because plunging leaves into liquid nitrogen was found to produce inconsistent results; possibly instant freezing produced insufficient membrane damage.

Apoplast samples varied in volume depending on the size and water potential of the leaf, but were usually between 50-150 μl, symplastic samples were always > 200 μl. Prior to the determination of osmolality 50 μl of sample were mixed with an equal volume of double distilled de-ionised water (samples < 50 μl were made up to 100 μl with de-ionised water).

Osmolality was determined with a Micro-Osmometer (Camlab, Cambridge) which was operated according to the manufacturer's instructions and was calibrated to 0 (de-ionised water) and 100 mOs.Kg^{-1} before and after use. To compensate for background contamination, 14 mOs was

deducted from readings for 50 μl samples and 18 mOs from samples < 50 μl. These values were obtained following deposition of 100 μl or 50 μl of de-ionised water onto lens tissue, from which it was possible to extract 50 and 15 μl respectively, the remaining fluid being absorbed by the tissue. Osmolality readings, adjusted for dilution, were converted to solute potential (at 20^0C) in bar by multiplying the value in $Os.Kg^{-1}$ by 24.37 (Cosgrove & Cleland, 1983).

2.3.8. Assessment of Hydraulic Resistivity

2.3.8.a. Calculation of Resistivity by Measurement of Water Flow and Water Potential.

The hydraulic resistance in palms was calculated with an analogue of Ohm's law as used by Black (1979), Nobel & Jordan (1983) and Koide (1985a.b.) :-

$$R = \frac{\Delta \Psi}{Flow}$$

R = resistance (MPa s m^{-3})

$\Delta \Psi$ = difference in water potential (Ψ) between 2 points (MPa)

Flow = water movement between 2 points (m^3 s^{-1})

To compensate for the reduction in leaf area in diseased palms, estimates of resistance were converted to resistivities (MPa s m^2 m^{-3}) by substituting flow in the above equation with flow per unit leaf area (m^3 m^{-2} s^{-1}).

Resistivities were calculated as follows :-

Resistivity soil to bulb = - (Ψ soil - Ψ bulb) ÷ plant transpiration rate per unit leaf area.

Resistivity bulb to leaf = - (Ψ bulb - Ψ leaf) ÷ leaf transpiration rate per unit leaf area.

Soil Ψ was measured with a Wescor HR33T dew point microvoltmeter (Wescor Inc, Utah, U.S.A.) used in the psychrometer mode. Bulb Ψ was estimated by covering a mid-canopy leaf with aluminium foil and a plastic bag on the night previous to the reading of water potential. The covered (nontranspiring) leaf provides an estimate of the water potential of the stem (bulb) to which it is attached (Black, 1979; Koide, 1985b). The midday water potential of this covered leaf and a comparable transpiring leaf (Ψ leaf) were measured with a pressure vessel (2.3.5)

Transpiration rate per unit leaf area was measured by 2 methods. In the first, whole plant weight loss (2.3.4.a.) over 3 hours at midday was combined with measurements of leaf area (2.3.1.b.). In the second method (Nobel & Jordan, 1983.), transpiration of a mid-canopy leaf, was approximated from calculations of stomatal and boundary layer conductance and the mole fraction gradient of water vapour (see Appendix 2). These measurements were immediately followed by assessment of leaf Ψ.

2.3.8.b. Calculation of Vascular Resistivity from Measurements of Xylem Diameter and Blockage.

The hydraulic resistivity of xylem vessels was calculated following the principles of the Hagen-Poiseulle law, as adapted by Dimond (1966) and Nobel and Jordan (1983). Thus :-

Thus $R = \frac{128\ l\ n}{\pi\ \Sigma\ d^4}$

R = resistance (MPa s m^{-3})

l = length of pathway, from petiole base to middle of leaf lamina (m)

n = viscosity of xylem sap (1 mPa s @ 20^0C, 0.8 mPa s @ 30^0C)

$\Sigma\ d^4$ = sum of the fourth power of all xylem diameters in a cross section of petiole (m^4)

To account for differences in leaf area, resistances were multiplied by the lamina area (m^2) to produce values of resistivity (MPa s m^2 m^{-3}).

Petiole xylem diameter measurements were made by microscopic examination of a petiole cross section half way along its lenght; vessels < 0.5μm in diameter were ignored. Σd^4 was calculated from measurements of all xylem vessels in 10 randomly selected vascular bundles and from the number of bundles in the petiole. Sap viscosity was calculated presuming sap temperature = air temperature.

Estimates of root system vascular resistivity were calculated as above, but with the following alterations. Measurements of root xylem diameter, 5mm below the stem, were taken for all vessels in 5 vascular bundles in 5

randomly selected roots from each plant. Σd^4 was calculated from counts of the number of bundles per root and roots per palm. Sap viscosity was estimated presuming sap temperature = soil temperature. Root system length was estimated from the mean length of the 5 measured roots, which presumed that roots are perfect cylinders and all water travels the whole length of the cylinder.

The percentage vascular occlusion in different parts of the palm was calculated from examination of all xylem vessels in the roots and petiole base and a sample of vessels (between 60 and 80) from the middle of the stem. Any vessel containing gels, gums or tyloses or with browning of cell walls was considered occluded. Estimates of vascular resistivity from soil to stem and stem to leaf were adjusted to take account of these blockages in the roots and petiole base respectively. In the palm stem, vascular tissue is highly interconnected and therefore estimates of occlusion in this region were not used in calculations of resistivity.

2.3.9. Statistical Analysis.

Data were analysed by use of Minitab (Minitab Inc, U.S.A.) or Statsease (B. Clarke, University of Nottingham) statistical programmes. Normality of data was tested by the n-scores method or by observation of the data in the form of histograms; homogeneity of variance was tested by Bartlett's test or by the Fmax method (Parker,1979). If the data were found suitable, an analysis of variance was performed, followed (as appropriate) by Bonferroni's

inequality test (an unplanned comparison of means) or Fisher's test (planned comparison of means).

If data were not suitable for analysis of variance, i.e. non-parametric, then a Kruskal-Wallis test was performed which, if significant, was followed by an S.T.P. nonparametric multiple comparisons test (Sokal & Rohlf, 1981) for unplanned comparisons, or repeated Mann-Whitney U-test (Seigel, 1956) for planned comparisons.

Examination of data in the form of ratios were made by Chi-squared analysis of contingency tables (Mead & Curnow, 1983) and, as appropriate, by Fisher's Exact test (Seigel, 1956).

3.0.

THE EFFECT OF *FUSARIUM* WILT DISEASE ON PALM PHYSIOLOGY

3.1. INTRODUCTION

Vascular wilt pathogens are characterized by their ability to invade the host vascular system and become systemically distributed, via the xylem elements, to all parts of the plant. Wilt diseases can display a variety of symptoms, but as the name suggests wilting due to water stress is their most characteristic feature and other symptoms may be direct or indirect consequences of pathogen induced water stress (Beckman, 1987).

The cause(s) of wilting have been the subject of some debate between proponents of the importance of water stress and an alternative theory (Gaumann, 1957, 1958) that pathogen-produced toxins could cause wilting. Such toxins were shown to damage the semi-permeability of mesophyll cells, and thus the ability of cells to remain turgid.

However, the toxin theory is now often discounted as a generalised explanation for wilting (Dimond, 1970; Beckman, 1987; Van Alfen, 1989) due to the strength of data supporting the involvement of water stress in disease symptoms and to a lack of evidence for membrane damage occurring *in vivo* before wilting or leaf chlorosis. On the contrary, most experimental evidence suggests that membrane damage does not occur. Thus wilting is reversible

and where measured, electrolyte leakage did not occur before symptom expression (Duniway, 1971a; Hall & Busch, 1971; MacHardy, Hall & Busch, 1974; Dey & Van Alfen, 1979; Goodwin, DeVay & Meridith, 1988a,b). It is perhaps unfortunate that this theory is still quoted in some standard texts (Agrios, 1988). However, toxins may be important in the suppression of host defence responses (Dimond, 1972; Beckman 1987; Van Alfen, 1989).

Renard (1976) suggested that toxins may have a role in symptom development in *Fusarium* wilt of oil palm, but the majority of authors have presumed or implied that water stress is the major cause of symptoms (Wardlaw 1950; Turner, 1981; Obueckwe & Osagie, 1989). However no experimental study of the host water relations of this disease has been conducted. Thus the experiments in this section were initiated to examine the physiology of infected and healthy palms and to establish the role of water stress in this disease.

To understand how water stress may be caused in diseased palms a basic knowledge of plant water relations is required, and consequently the current concepts of the water relations of healthy plants will be summarised before reviewing the water relations of plants infected with vascular pathogens.

3.1.1.The Thermodynamic Theory of Plant Water Relations

Approximately 99% of water taken up by plant roots is lost through evapotranspiration and the remainder is mostly used for cell expansion (Newman, 1976). This

movement of water from the soil via the plant to the atmosphere can be conveniently described by the concepts of thermodynamics. Thus, water will move from A to B if B represents a lower energy level (Slatyer, 1967; Passioura, 1982; Boyer, 1985). The energy level of water is called the water potential and is reduced by solutes, colloids, and surfaces, increased by elevation and increased or decreased by hydrostatic pressure or suction. In plant cells the effects of colloids and surfaces are regarded as negligible and elevation is only important in tall trees. Thus, in simplified form the water potential of a typical leaf cell will be the sum of its solute (osmotic) and pressure (turgor) potential and is usually expressed algebraically as force per unit area (1 Megapascal = 10 Bar = 145 pounds per square inch). In the following example, of a typical leaf cell, total water potential of -1.5 MPa is mostly due to dissolved solute in the cell symplast, which is partly offset by positive cell pressure (turgor).

Cell water potential = Solute potential + Pressure potential

- 1.5 MPa = - 1.8 MPa + 0.3 MPa

In contrast in xylem vessels, where solute potential is negligible, water potential is controlled by pressure potential which in a transpiring plant is negative :-

Xylem water potential = Solute potential + Pressure potential

- 1.0 MPa = - 0.05 MPa + - 0.95 MPa

In these examples, water would move from the xylem towards the leaf cell which has a lower total water potential and therefore represents a lower energy level. Therefore the direction of water movement is controlled by the water potential gradient and this explains why water moves from the soil through the plant to the air:-

Typical water potential values (Baker 1984)

Ψ Soil	- 0.1	MPa
Ψ Root	- 1.0	MPa
Ψ Leaf	- 1.5	MPa
Ψ Air	- 100.0	MPa

Ψ indicates water potential

However, water movement through plants is not only controlled by differences in water potential but also by the resistance to water movement in the flow path. Thus, the flow from one part of a plant to another depends on the difference in water potential divided by the resistance. Under steady state conditions (ignoring movement of water stored in tissue), water flow from soil to root to leaf to air is the same. By inserting the values of water potential into the equation below it is possible to calculate the relative resistance of different sections of the pathway. Thus :-

$$\text{Water flow} = \frac{\Delta\Psi}{R} = \frac{\Psi \text{ soil} - \Psi \text{ root}}{R1} = \frac{\Psi \text{ root} - \Psi \text{ leaf}}{R2} = \frac{\Psi \text{ leaf} - \Psi \text{ air}}{R3}$$

Relative resistance:- R1 = 1.8, R2 = 1, R3 = 197

Obviously, by far the greatest resistance occurs between the leaf and air. This is a variable resistance

controlled by stomatal movement, and plants can control water movement by adjusting stomatal resistance. The second largest resistance (R1) occurs between the soil and the root and is largely caused by the endodermal cells through which all water must flow. Within the roots and stem most water movement occurs through xylem tissue and this is due to the very low resistance of the lumen of mature, non-living, xylem cells. Resistance in xylem cells is governed by sap viscosity, by the permeability of pit and perforation plates between xylem elements and by vessel diameter. The significance of the latter is shown by the Hagen- Poiseuille equation which reveals that a doubling of vessel diameter results in a 16 times increase in sap flow (Dimond, 1966; Nobel & Jordan, 1983). Within the leaf, water moves from xylem elements to mesophyll cells along gradients of solute and matric potential.

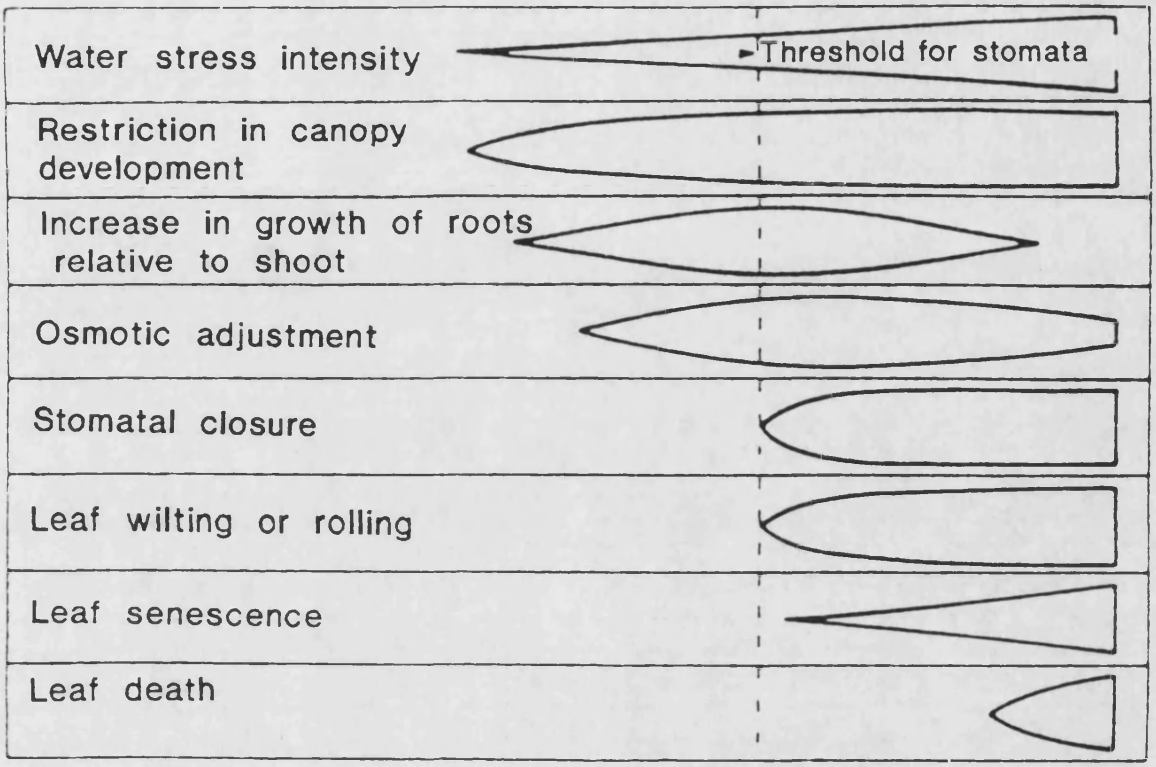
As mentioned previously, stomatal movement provides an important mechanism for controlling plant water loss and stomatal closure can be induced by water stress. However it is not the only reaction to this stress and it is necessary to outline some other responses of non-infected plants before discussion of the water relations of plants with wilt diseases.

3.1.2. Plant Responses to Water (Drought) Stress

If water stress is defined as a reduction in water availability below the level required for maximum growth, then most plants are water stressed for much of their

lives (Reid & Wamble, 1985). The range of adaptive responses to water stress have been the subject of much experimentation and many reviews (Hsiao, 1973; Boyer, 1973; Bradford & Hsiao, 1982; Schulze & Hall, 1982; Morgan, 1984; Boyer, 1985; Reid & Wamble, 1985; Dale, 1988) and are summarized in Figure 3. However, whilst the responses of plants have been well described the exact causes of these changes still remain controversial. For example, stomatal closure and reduced growth could be caused by a decrease in leaf water potential or by chemical signals from the roots (Kramer, 1988; Passioura, 1988; Schulze, Gollan & Schurr, 1988; Boyer, 1989; Davies & Zang, 1991).

Figure: 3. Generalized Response of Plants to the Gradual Development of Water Stress. (from Bradford & Hsiao, 1982)



The starting point of each band represents the threshold water stress for eliciting the response and the band width indicates the relative magnitude of the response. This is a very generalised diagram and species can vary considerably in thresholds and which responses occur first.

In response to continuous mild water stress plants produce fewer and/or smaller leaves (thus reducing water loss), increase root growth relative to shoots and may accumulate solutes. This latter osmotic adjustment means that leaf cells can remain turgid at lower water

potentials. Increasing water stress, or stress that occurs too quickly for the above adjustments to occur, will result in stomatal closure, which decreases water loss but also reduces CO_2 assimilation; another cause of reduced growth.

Under more acute stress, further reductions in canopy size may occur through premature senescence or abscission of older leaves. Leaf water potential may decline to the point where the vacuole no longer exerts pressure against the cell wall (turgor < 0), the cells lose rigidity and the leaf collapses. Such wilting can be reversible, with recovery overnight, or if this is not possible then leaves are said to be permanently wilted.

Severe water stress can also result in vaporisation of water in the xylem vessels, which may follow the aspiration of microbubbles of air through pit pores. The resultant gas-filled (embolised) vessel is no longer functional but if xylem pressure becomes positive then recovery (water filling) is possible (Milburn, 1973a,b; Tyree & Sperry, 1988). The frequency and extent to which embolisms occur under natural conditions remains unclear and this is partly due to their transient nature and the difficulty of detection (Zimmermann, 1983). The comparative susceptibility of smaller shoots of woody plants to embolism compared to the main stem has led Zimmermann (1983) and Sperry (1986) to speculate that under conditions of severe water stress embolisms result

in the hydraulic isolation of these shoots and therefore a reduction in water loss.

To summarize, plants can react to water stress in a number of ways depending on the species, variety, and the severity and speed with which it occurs. Importantly, several significant changes occur at a level of stress below that needed to cause stomatal closure. These adaptations allow plants to increase water uptake, reduce water loss and function at lower water potentials.

3.1.3.Water Relations of Palms

Having reviewed general plant responses to water stress, it is necessary to summarize our knowledge of the water relations of palms and their responses to drought.

Palm vascular anatomy has some unusual characteristics which have important implications for their water relations. Palm trunks have only two growing points, one at the top of the stem that produces new leaves and infloresences, and another at the stem base producing thousands of roots (Hartley, 1988; Tomlinson, 1990). In the trunk there is no secondary thickening and vascular elements are merely extended at the trunk apex as the palm grows. The irreplaceable nature of these trunk vascular elements could make the xylem system vulnerable to damage, but a complex interconnecting network of vascular bundles ensures that any point in the pathway can be reached by innumerable alternative routes. Thus injuries to the palm stem rarely disrupt water flow (Zimmermann & Brown, 1971).

Studies of the vascular anatomy of the palm *Rhapis excelsa* (Zimmermann & Sperry, 1983; Sperry, 1986) have demonstrated that in the trunk water movement occurs in long, wide diameter, xylem vessels of low resistance. However in the leaf base there is a constriction caused by small metaxylem vessels of low conductance, which results in a sharp drop in xylem water potential from the trunk to the petiole. Therefore, under conditions of severe water stress cavitation is confined to the leaf and the function of the more important and irreplaceable stem xylem system is preserved.

There has been no comparable study of the vascular system of oil palm, but based on the similarity of the leaf trace structure, Zimmermann & Sperry (1983) concluded that the xylem system of other palms would be correspondingly adapted to water stress.

Water stress is regarded as one of the main limiting factors for oil palm growth (Ochs & Daniel, 1976), especially during the 2 to 4 month dry season in West Africa, and there have been several studies of the response of oil palm to drought.

Under conditions of water deficit, oil palm restricts water loss by stomatal closure, which can occur for a few hours at midday (Rees, 1961) or for several weeks (Ochs & Daniel, 1976). During prolonged periods of drought, water loss is also reduced by the failure of newly developed leaves to unfurl (Hartley, 1988).

In experiments on the effect of drought on young clonal palms, Barrett, Smith & Jones (1985) and Potulski, Barrett, Smith & Jones (1986) demonstrated that palms responded by a reduction in total leaf area whilst maintaining the same transpiration rate per unit leaf area as well watered plants. The reduction in leaf area was due to reductions in leaf production, premature senescence of older leaves and/or reduced number or size of new leaves (Smith, 1992, pers comm). In subsequent experiments, reductions in stomatal conductance were also observed (Barrett, Potulski & Smith, 1987; Potulski, Smith & Barrett, 1988).

Non-stomatal reductions in photosynthetic ability in response to water stress have been reported (Corley, 1976; Potulski, Barrett & Smith, (1988), although no evidence of osmotic adjustment was observed (Barrett *et al.*, 1987). Furthermore, Smith (1989) demonstrated that although stomatal conductance was primarily controlled by soil water availability, under conditions of adequate water supply, transpiration could be reduced by a high vapour pressure deficit *e.g.* high air temperature and low humidity.

In summary, oil palm responds to water stress by reductions in leaf area and transpiration. The vascular anatomy of palms exhibit features which make them potentially vulnerable (irreplaceable stem xylem) and tolerant (highly interconnected stem xylem and constant

production of new roots from the stem base) to disruption by vascular pathogens.

3.1.4. Water Relations of Plants with Vascular Wilt Disease

As previously mentioned, wilting is a common symptom in this group of diseases and is generally attributed to water stress. This section will outline:-

a) The evidence that plants suffering from wilt diseases are water stressed

b) Possible causes of water stress

c) The relationship between symptoms and water stress

3.1.4.a. The Evidence for Water Stress in Plants Infected with Vascular Pathogens

There are many reports that provide indirect evidence for water stress in plants infected with vascular pathogens. Dimond & Waggoner (1953) and Duniway (1971a) demonstrated that if flaccid leaves were removed from *Fusarium* infected tomato plants and their petioles placed in water, than full recovery of turgor was possible; similar results were reported for *Verticillium* wilt of tomato (Threlfall, 1959). Duniway (1971b) also observed reversible wilting, based on the removal of discs from flaccid leaves of *Fusarium* infected tomato plants which regained turgor when floated on water. Dey and Van Alfen (1979) reported a similar response with leaf discs from alfalfa infected with *Clavibacter insidiosum*.

Further indirect evidence for the role of water stress in symptom development is provided by the failure of

wilting to occur if infected plants are grown in conditions of 100% relative humidity, as reported by Gottlieb (1944) with *Fusarium* wilt of tomato and in *Xylella* wilt of grapevine (Goodwin *et al.*, 1988a).

Likewise, observations of reduced transpiration in tomato plants infected with *Fusarium* wilt (Dimond & Waggoner, 1953; Duniway, 1971b; and Duniway & Slatyer, 1971) are also indicative of water stress in diseased plants. These reductions in transpiration have been reported in many other wilt diseases including *Fusarium* wilt of banana (Page, 1959), and *Verticillium* wilt of tomato (Threlfall, 1959), potato (Harrison, 1971) and chrysanthemum (MacHardy, Busch & Hall, 1976), and in bacterial wilt of grapevine (Goodwin *et al.*, 1988b) and alfalfa (Dey & Van Alfen, 1979).

Indirect evidence of dehydration in wilt diseases is also supported by more direct experimental measurement of plant and leaf water status. Thus, Harrison (1970, 1971) in a study of *Verticillium* wilt of potato, observed a marked reduction in leaf relative water content (RWC) in infected plants before wilting occurred, and a similar reduction in leaf RWC was reported in *Verticillium* wilt of chrysanthemum at the time of wilting (MacHardy *et al.*, 1974, MacHardy *et al* 1976).

An alternative and more sensitive estimate of leaf water stress can be obtained by use of a Scholander pressure chamber (Boyer, 1967). It is possible with this

equipment to estimate leaf water potential by placing a leaf in the chamber with only the petiole protruding and increasing the pressure until fluid first appears at the cut end of the petiole. The pressure is then presumed to be equal and opposite to the leaf water potential when the leaf was attached to the plant. As a consequence, the more water stressed the leaf, the greater the pressure that is required to force fluid from the petiole.

Using this technique, Duniway (1971a) showed that wilting of *Fusarium* infected tomatoes was associated with reduced leaf water potential and that leaves from diseased and water stressed uninoculated plants wilted at similar water potentials. In a study of alfalfa bacterial wilt, Dey and Van Alfen (1979) also demonstrated a significant reduction in xylem pressure potential in infected plants before wilting occurred and leaves from diseased plants wilted at a lower water potential than those from healthy plants, which indicated an adjustment to disease-induced water stress. Comparable reductions in water potential of diseased leaves were also reported by Goodwin *et al* (1988a) in a study of *Xylella* wilt of grapevine, in this study water potential was assessed by psychrometry.

The evidence so far indicates that vascular wilt diseases are associated with varying degrees of host water stress; however, how could such water stress occur?

3.1.4.b. Causes of Water Stress in Diseased Plants

An increase in water loss from diseased plants is one possible cause of wilting and this has been demonstrated in almonds (*Prunus amygdelus*) infected with *Fusicoccum amygdali* (not a vascular pathogen), where the translocatable toxin, fusicoccin, has been shown to increase transpiration by stimulating stomatal opening (Ballio 1978). There have been a few reports of increased transpiration in vascular wilt diseases (Threlfall, 1959; Scheffer & Walker, 1953) but such rare observations are often not statistically significant and only occur at the early stages of disease.

The generally accepted cause of wilting is a reduction in water supply to the leaves. Early support for this theory was provided by studies of dye ascent through the xylem of diseased plants. Beckman, Kuntz, Riker & Berbee, (1953), in a study of *Ophiostoma* wilt of oak observed a sudden drop in the flow of radioactive rubidium to leaves two days before initial wilting occurred. Similar observations were noted in tomato infected with both *Fusarium* (Dimond & Waggoner, 1953, Scheffer & Walker, 1953) and *Verticillium* (Threlfall, 1959) wilts, where a decreased flow was correlated with the degree of wilting. These reductions in flow only provide indirect evidence for an increase in xylem resistance since decreased transpiration could also be responsible for these observations.

More direct attempts to measure xylem resistance have been made by measuring the flow through excised plant

sections under conditions of constant pressure. Duniway (1971b) demonstrated that in *Fusarium* infected tomato plants there was a 3-30 times increase in stem resistance when wilting occurred, but no increase in root resistance was observed. Duniway also calculated xylem resistance from differences in water potential, and again concluded that wilting was due to an increase in xylem resistance, with the greatest increase occurring in the petiole (Duniway 1971b). Similarly, in a pressure/xylem flow study of *Verticillium* wilt of tomato, Street and Cooper (1984) found that petiole resistance was greatly increased in diseased plants and this occurred before visual symptoms were apparent. Similar results have also been reported in *Fusarium* wilt of banana (Page, 1959), *Verticillium* wilt of tomato (Threlfall, 1959), *Corynebacterium* wilt of alfalfa (Dey & Van Alfen, 1979) and *Xylella* wilt of grapevine (Goodwin *et al.*, 1988a).

The actual site of vascular blockage and vascular architecture of the host can have an important influence on xylem conductance. Thus Dimond (1966) suggested that in tomato, occlusion of half the stem vascular system would not markedly increase overall resistance to flow, due to the large diameter and interconnecting nature of xylem in the stem. However, in the leaf petiole a similar level of occlusion would have a much greater effect due to the narrow diameter and parallel design of the xylem in this region. These theoretical observations have been borne out

by studies of tomato wilts (Duniway, 1971a,b; Street & Cooper, 1984).

Therefore, a reduction in leaf water supply due to an increase in xylem resistance is a common feature of vascular wilt diseases, but the mechanisms proposed for reduced xylem conductance have been varied and sometimes controversial.

Beckman (1964) suggested that host water stress was due to the defence responses of plants to damage and microbial invasion, which generally involved xylem vessel occlusion with gels, gums and tyloses. He proposed that in a susceptible plant, these responses form too late to prevent systemic pathogen spread, which is consequently followed by systemic vascular occlusion. His suggestions, which were largely based on observations of *Fusarium* wilt of banana, are supported by observations in *Verticillium* wilts of chrysanthemum (Robb, Busch & Lu, 1975; Douglas & MacHardy, 1981) and cotton (Misaghi, DeVay & Duniway, 1978), *Fusarium* wilt of mimosa (Phipps & Stipes, 1976) and *Ophiostoma* wilt of oak (Stuckmeyer, Beckman, Kuntz & Riker, 1954).

The mechanism of vascular occlusion can vary depending on the host; gels predominate in *Fusarium* and *Verticillium* wilt of peas whilst tyloses form the major occluding mechanism in tomato (Bishop & Cooper, 1983). Alternatively, in a survey of a range of *Verticillium* wilt diseases, Robb, Smith & Busch (1981) concluded that leaf

wilting may be primarily due to the coating of xylem walls with a lipid-rich material, possibly suberin (Street, Robb & Ellis, 1986). Coating of xylem walls, pit cavities and membranes has also been observed in an electron microscopy study of *Fusarium* and *Verticillium* wilts of tomato (Bishop & Cooper, 1983).

Water movement can also be prevented if only pit membranes are obstructed. This may occur if pathogen produced enzymes (either polygalacturonases or pectic lyases) degrade the middle lamella in the exposed primary wall in pit membranes (Cooper & Wood, 1980). Pit membranes may also be obstructed by pathogen produced high molecular weight metabolites, in particular, the extracellular polysaccharides produced by bacterial pathogens have been shown to obstruct pit pores at very low concentrations (Van Alfen & Turner, 1975; Van Alfen & Allard-Turner, 1979; Van Alfen, McMillan & Wang, 1987). Xylem occlusion may also be directly caused by the pathogen itself, as suggested by Mollenhauer and Hopkins (1976), who observed bacterial aggregates in the xylem of grapevines infected with *Xylella*.

Xylem can also be disrupted by embolisms. Zimmermann & McDonough (1978) and Sperry & Tyree (1988) proposed that the first penetration of a pathogen into a xylem vessel under negative pressure would result in cavitation (embolism). Zimmermann (1983) suggested that the production of tyloses, gels and suberization of xylem walls was a consequence of such events. Embolisms can be

very difficult to detect, because as soon as a plant section is removed for examination, severed xylem vessels become air-filled and embolised xylem may refill with water at atmospheric pressure. However, Newbanks, Bosch & Zimmermann (1983) were able to detect xylem disfunction before the production of visible occlusions in elm seedlings infected with *Ophiostoma ulmi* and they attributed this to the formation of embolisms as vessels were penetrated.

However, in other diseases where the first penetration of xylem occurs in the roots, embolisms may not occur because here critical negative pressures in the xylem are uncommon. Alternatively, penetration of hyphae from xylem vessels into adjacent xylem parenchyma could induce embolisms especially if the vessel was under sufficient negative pressure, as may be the case in aerial tissue (Shi, Mueller & Beckman, 1991).

Furthermore, Sperry and Tyree (1988) suggested that chemical changes in the xylem fluid in diseased plants could make embolism more likely through undamaged pit pores. Thus, the addition of millimolar quantities of oxalic acid and calcium chloride to the xylem fluid of sugar maple resulted in significant increase in the xylem pressure at which embolisms occurred. They ascribed this effect to increased middle lamella elasticity leading to transient pore widening as xylem pressures decreased; Van Alfen (1989) suggested that pathogen produced cell wall degrading enzymes may have a similar effect.

Vascular occlusion in diseased plants caused by embolisms is largely theoretical and if it were a common occurrence pathogens would exist only in non-functional vessels (unless embolisms were regularily "repaired" overnight). This is incompatible with observations of systemic spore movement (Jones, 1985). However, Zimmermann (1983) regarded the evidence for spore movement in the transpiration stream as circumstantial. Alternatively, the success of vascular pathogens may actually depend on their ability to enter xylem vessels under negative pressure without allowing the entry of microbubbles (Jones, 1985; Newbank *et al.*, 1983).

In summary, vascular wilt diseases are associated with host water stress and this is due to reduced xylem conductance, which results from a variety of factors which may act in isolation or in combination. It is now necessary to assess the degree to which the symptoms of this group of diseases can be attributed to water stress.

3.1.4.c. The Relationship Between Disease Symptoms and Water Stress

Vascular wilt pathogens can induce a range of disease symptoms depending on the host/pathogen combination and environmental conditions. Typical symptoms include chlorosis and necrosis of leaves and growth responses such as epinasty, the production of adventitious roots and stunting. However, as the name suggests, this group of diseases is generally associated with leaf wilting and there now seems little doubt that this symptom, at least,

is due to water stress, caused by a reduced water supply to leaf cells.

The link between water stress and leaf chlorosis and necrosis is less clear than with wilting. Thus, although these symptoms frequently occur in water stressed uninfected plants, in some wilt diseases the pattern of leaf chlorosis is indicative of premature senescence rather than water stress (Talboys, 1968). Unilateral leaf chlorosis has been observed in a number of diseases, but Corden and Chambers (1966) attributed this to waterproofing of the infected xylem on one side of the leaf, so that lateral movement of water from xylem on the other side of the petiole was no longer possible.

In *Verticillium* wilt of hop and raspberry, characteristic 'tiger-striping' can occur, which Talboys (1968) believed could only be explained in terms of water stress if occlusion took place in the terminal leaf tracheids. Indirect evidence to support this theory comes from a study of bacterial wilt of alfalfa, where the smallest of three pathogen-produced extracellular polysaccharides passed through pit membranes and accumulated in leaf blades. Here it was able to induce wilting, possibly by blocking cell wall capillaries (Van Alfen *et al.*, 1987).

MacHardy *et al* (1974; 1976) presented evidence for the localised leaf symptoms in *Verticillium* wilt of chrysanthemum resulting from water stress, which could be

attributed to localized xylem plugging and water proofing (Robb *et al.*, 1975; Douglas & MacHardy, 1981). A similar conclusion was reached by Misaghi *et al.*, (1978) in a study of limited leaf symptoms in *Verticillium* wilt of cotton.

However, Pegg (1989) concluded that vascular occlusion would not explain the stomatal behaviour of 'tiger stripe' areas in hop, where stomata open wide and remain so regardless of environmental conditions. He suggested this symptom may be induced by a toxin, and that water stress alone could not cause leaf curling in cotton wilt or adventitious leaf production in Dutch elm disease. But, Dale (1988) suggested that the failure to simulate some symptoms may reflect the difficulty of applying a realistic mild water stress, over a long period, to pot grown plants.

In summary, leaf chlorosis and necrosis may not always be explained in terms of general host water stress, especially when it is confined to clearly defined regions of a leaf. This may reflect the difficulty of measuring localised water stress. However, with the development of the pressure probe (Husken, Steudle & Zimmermann, 1978; Cosgrove & Cleland, 1983), which allows the turgor of individual cells to be assessed, it may be possible to evaluate the degree of water stress in such localized areas.

Another common symptom of wilt diseases (at least where host death does not occur rapidly) is a reduction in growth, as reported in *Verticillium* wilt of tomato (Threlfall, 1959; Selman & Pegg, 1957), potato (Harrison, 1971) and cotton (Tzeng, Wakeman & DeVay, 1985), Pierce's disease of vines (Goodman *et al.*, 1988b) and bacterial wilt of alfalfa (Dey & Van Alfen, 1979). In all of the above, stunting was attributed to or correlated with mild host water stress. The exception was Selman & Pegg (1957), and Pegg & Selman (1959) later suggested that reduced growth could be due to toxins or the accumulation of IAA in diseased tissue.

Stunting may also result from reduced photosynthesis, following stomatal closure, as noted in *Verticillium* wilt of cotton (Mathre, 1968; Hampton, Wullschleger & Oosterhuis, 1990) and *Fusarium* wilt of tomato (Duniway & Slatyer, 1971). In Pierce's disease of vines, reduced photosynthesis could not be totally explained by increased stomatal resistance, and toxins rather than water stress were suggested as a possible cause (Goodman *et al.*, 1988b)

In summary, in nearly all cases where host water relations have been studied there is a reduction in leaf water potential before or coincident with symptom development which is sufficiently clear to suggest a cause and effect relationship. However, in some diseases water stress may not have a direct role in symptom development, but even in these cases, symptoms can be expected to occur against a background of host water stress.

For a variety of reasons, studies of vascular diseases usually involve artificial and massive inoculation of susceptible hosts that quickly produce clear and extreme symptoms. In such plants very large increases in vascular resistance have been reported (Duniway, 1971b; Street & Cooper, 1984). Hall and MacHardy (1981) stated that since root and vascular resistance are 2 or 3 orders of magnitude less than stomatal resistance that vascular resistance would have to increase by this magnitude before exerting any significant effect on transpiration

However, relatively minor increases in xylem resistance will have a significant effect on plant physiology. Thus, using the example of water potentials and relative resistances on page 34, if R2 (xylem resistance) were to double, then in order to maintain the same transpiration flow, the water potential gradient between the root and leaf must also double *i.e.* the leaf water potential would have to fall from -1.5 to -2.0 MPa. This is a significant reduction in leaf water potential and could be expected to be associated with stunting and osmotic adjustment. Importantly, any further reduction in leaf water potential would induce progressive stomatal closure, thus reducing the transpiration flow and changing the whole equation. This means that further increases in R2 would have progressively less effect on leaf water potential.

Therefore, although artificial inoculation of experimental plants is necessary for the production of uniformly infected plants, this procedure can result in

the development of unrealistic symptoms. However, in *Fusarium* wilt of oil palm, inoculated seedlings and plantlets take several months to develop symptoms, and therefore this disease provides a good model system for the study of host physiology under what may be a more natural level of infection.

3.2 RESULTS

Several authors have presumed that water stress is the main cause of symptom development in *Fusarium* wilt of oil palm (Wardlaw, 1950; Turner, 1981; Obueckwe & Osagie, 1989). However, since no measurements of host water stress or physiology have ever been performed, this theory remains unsupported. The experiments in this section were designed to examine the water relations, development and physiology of healthy and diseased palms.

3.2.1. Symptom Development and Physiology of Inoculated Seedlings

Palms of a known susceptible cross (704/5 x 704/5) were propagated and inoculated at the 2 leaf stage with the high dose of inoculum (2.1 & 2.3). At monthly intervals, measurements of leaf area, leaf chlorosis and transpiration by weight loss were performed (2.3.1.b, 2.3.2.a & 2.4.a). At intervals, stomatal resistance was measured with a porometer and photosynthesis and stomatal conductance with an I.R.G.A. (2.3.4.b,c).

Infected palms had a smaller total leaf area (< 50%) than controls (Figure 4 & Table 1), this was due to the stunting of new leaves with no significant reduction in the number of leaves produced. Thus, 176 days post inoculation, mean leaf number was 9.4 for controls and 9.1 for inoculated palms. At this stage, canopy size in infected palms was further reduced (but by only 7%) by the premature senescence of older leaves (Figure 4).

There was a large reduction (> 60%) in the transpiration of diseased plants, which was primarily due to leaf stunting, and there was also a 28% reduction in transpiration per unit leaf area (Table 1) which was associated with an increased stomatal resistance (Table 2).

Table 1: Effect of Infection on Seedling Leaf Area and Transpiration

	Green Leaf Area* ($mm^2 \times 10^3$)	Transpiration over 48 hours (ml)	Transpiration Rate (ml per $mm^2 \times 10^3$)
Control	111.5	67.6	0.611
Inoculated	61.5	26.7	0.438

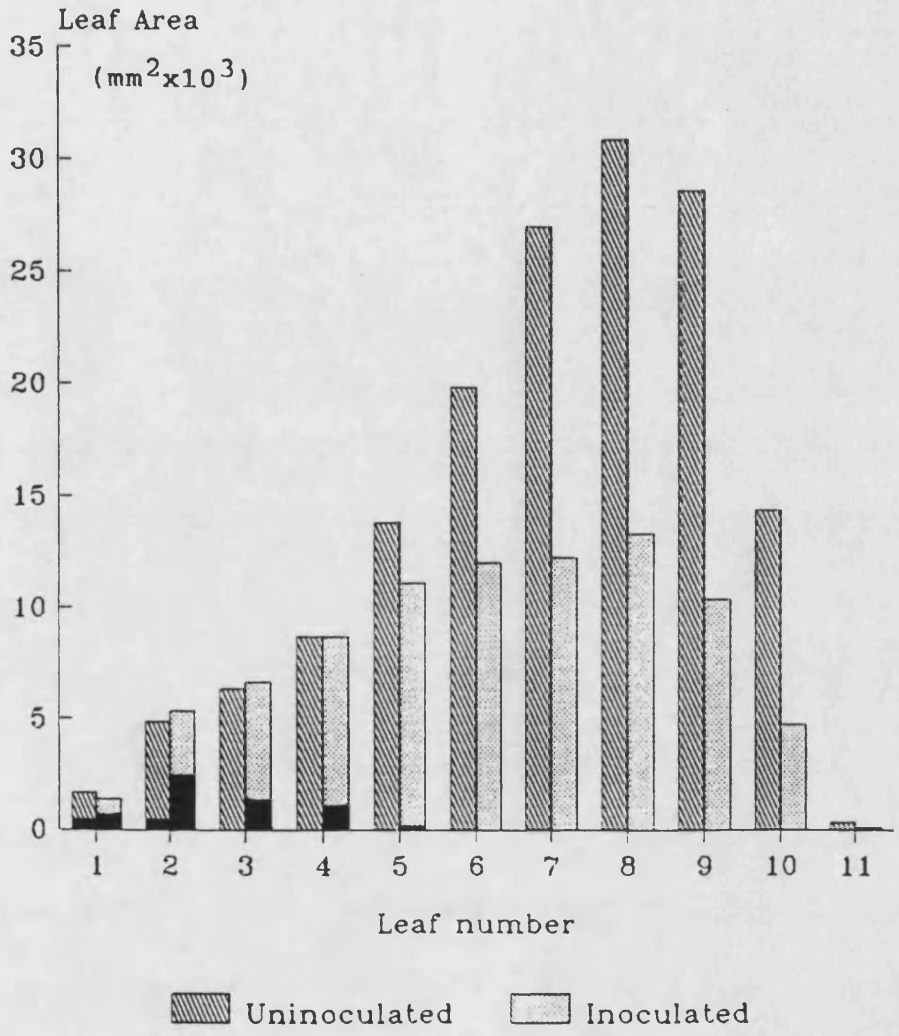
Values are the mean of 20 replicate plants, 140 days post inoculation.
All values for inoculated plants are significantly different to controls ($P < 0.01$, Mann Whitney U-Test).
* Total leaf area minus chlorotic leaf area.

Table 2: Effect of Infection on Seedling Stomatal Resistance

	Stomatal Resistance (s/cm)					
	Time of Day (GMT)					
	7.00	10.00	11.30	13.30	15.30	17.00
Control	11.3a	3.63a	3.95a	4.55a	6.00a	14.61a
Infected	8.75a	4,96a	7.21b	8.35b	7.41a	18.86a

Porometer readings were performed on leaves in mid-canopy and values are the mean 3 stable readings from 20 replicate plants, 142 days post inoculation.
Within each column, figures with the same letter are not significantly different ($P > 0.05$, Mann Whitney U-Test).

Figure 4: Effect of Inoculation on Leaf Area of Seedling Palms



Black areas represent chlorotic and necrotic tissue.
Values represent the mean of 20 plants, 176 days post inoculation.
Leaf 1 is the oldest leaf, each successive leaf is larger than the previous; leaves 9-11 are not fully developed.

I.R.G.A. measurements, which could only be performed on one occasion, demonstrated that when stunting first became apparent there was a 40% reduction in both stomatal conductance and photosynthesis and a 9% increase in calculated internal CO_2 (Table 3)

Table 3: Effect of Infection on Seedling Stomatal Conductance and Photosynthesis at Midday

	Stomatal Conductance (mMol m^{-2} s^{-1})	Photosynthesis (μMol m^{-2} s^{-1})	Leaf Internal CO_2 concentration (ppm)
Control	153.8a	5.623a	296.4a
Infected	94.1b	3.444b	323.1a

Readings were performed on leaves in mid-canopy and values are the mean of 14 replicate plants, 88 days post inoculation.
Within each column, values with the same letter are not significantly different ($P > 0.05$, Mann Whitney U-Test).

In experiments on the effect of water stress on oil palm physiology, Smith (1989) demonstrated that photosynthesis was only reduced when stomatal conductance fell below 100 mMol m^{-2} s^{-1}. If the 7 inoculated plants in this experiment with stomatal conductances < 100 mMol m^{-2} s^{-1} were excluded (no control plants had values < 100 mMol m^{-2} s^{-1}) then the mean stomatal conductance, photosynthesis and internal CO_2 values for inoculated palms were indistinguishable from controls (respectively 145, 5.91 & 294). In the 7 plants with low stomatal conductance (mean 43) there was a significant reduction in photosynthesis (mean 0.979) and an increase in internal CO_2 (mean 352).

Although this experiment demonstrated reductions in rates of transpiration and photosynthesis in infected palms, this was only indirect evidence of water stress. Therefore the experiment was repeated with more precise

observations of plant physiology and using clonal plants to reduce variability.

3.2.2. Development and Physiology of Inoculated UF4 Clonal Palms.

In the previous experiment, 2 distinct types of leaf symptom were observed in diseased palms namely, stunting of new leaves and chlorosis of older leaves. Some plants became severely stunted with little or no leaf chlorosis, and to a lesser extent, chlorotic plants with no stunting were also observed. It was therefore possible that these 2 symptoms did not have a common cause.

Previous experiments with UF4 clonal palms indicated that for several months after inoculation stunting was often the only symptom, and at a later stage this could be accompanied by leaf chlorosis.

A series of experiments was therefore initiated to study the water relations and physiology of infected palms at 2 stages. Firstly, when stunting was the major symptom and secondly when stunting was accompanied by significant levels of leaf chlorosis. Therefore, observations in this section were not based on a random selection of infected palms, but on plants that were selected as good examples of either stage of symptom development.

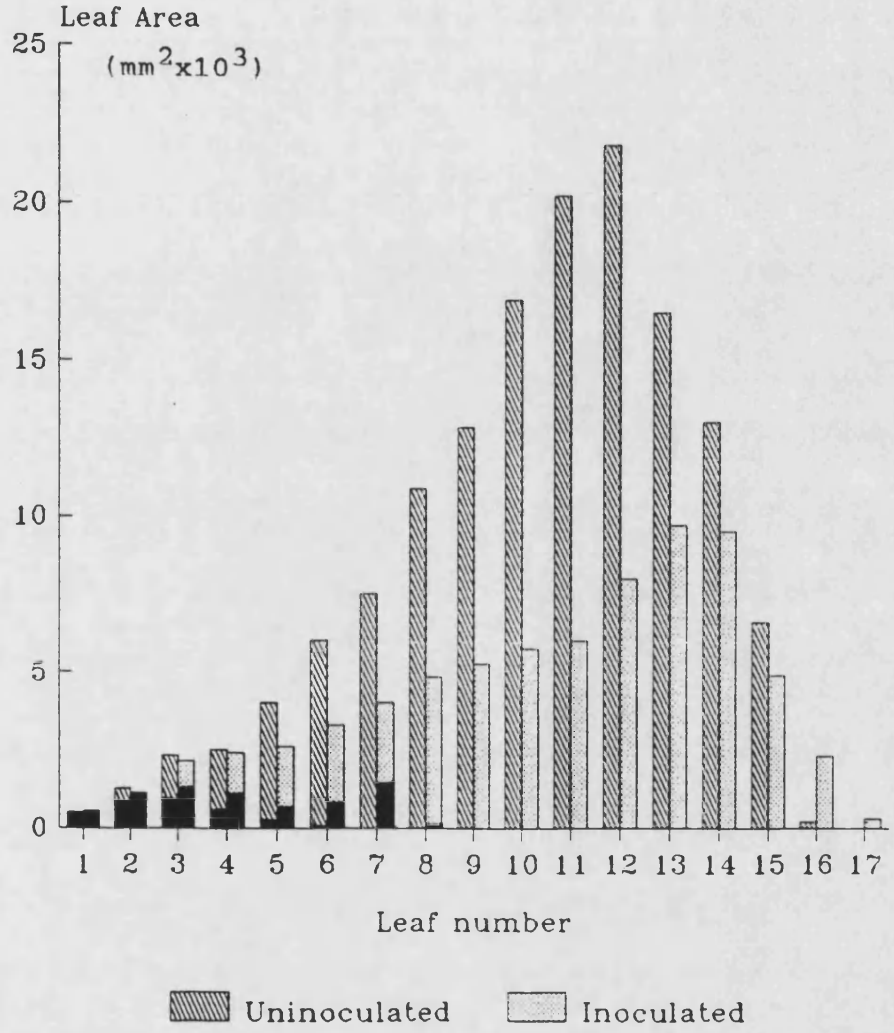
Clonal plantlets were hardened-off and grown as previously described (2.1), then inoculated and assessed for disease symptoms as in the previous section. In addition, leaf water potential was measured (2.3.5).

3.2.2.a. Stunted palms.

Infection resulted in a 50% reduction in the total canopy size, which was due to stunting of new leaves (Figure 5). This was also reflected in a reduction in total transpiration (Figure 6), but when reduced leaf area was taken into account, there was no reduction in transpiration per unit leaf area (Figure 7). This suggested that (unlike in the previous seedling experiment) inoculated plants were not water stressed.

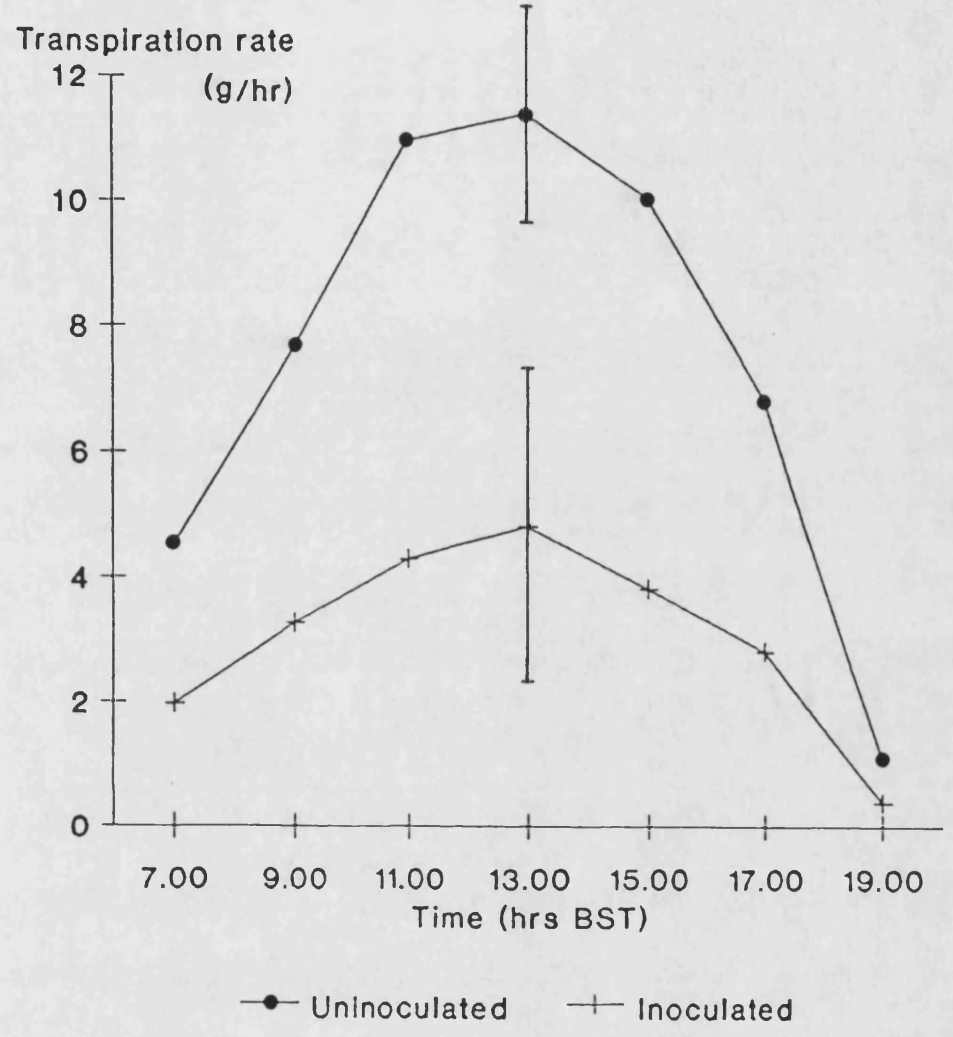
However, measurements of leaf water potential (Table 4) demonstrated that by 14.00h stunted leaves from inoculated plants were significantly more water stressed than controls, but that at dawn (07.00h) the water potential of inoculated plants was the same as controls. There was a very strong correlation between dawn and midday water potential values for inoculated plants but not for controls (Table 4). Thus stunted palms were able to recover from water stress overnight and the correlation between readings suggested that the degree of recovery reflected the severity of water stress for each plant.

Figure 5: Effect of Inoculation on Leaf Area of UF4 Clonal Plants



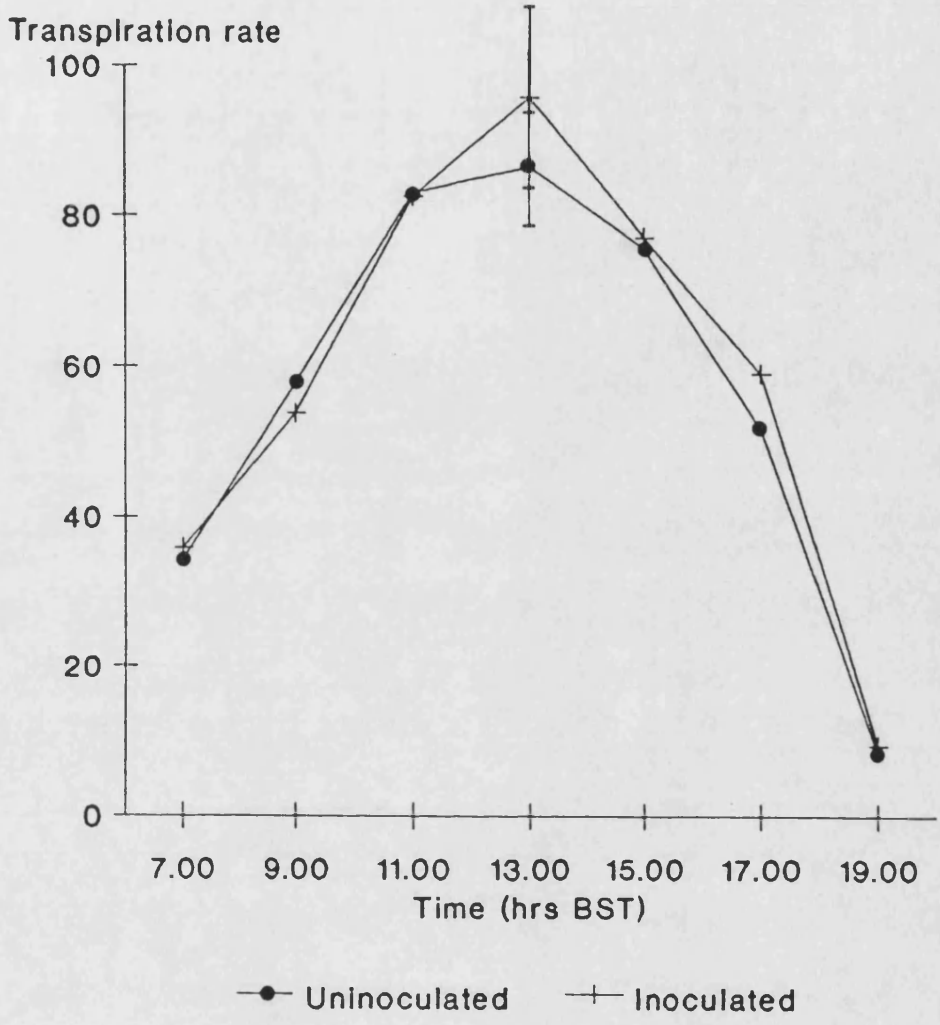
Black areas represent chlorotic and necrotic leaf tissue.
Values are the mean of 8 plants, 210 days post inoculation.
Leaf 1 is the oldest leaf, each successive leaf is larger; leaves 13 onwards are not fully developed

Figure 6: **Effect of Inoculation on Transpiration of UF4 Clonal Palms**



Values represent the mean of 8 replicate plants, 210 days post inoculation.
Bars represent 95% confidence limits.

Figure 7: Effect of Inoculation on Transpiration, Per Unit Leaf Area, for UF4 Clonal Palms.



Transpiration rate per unit green leaf area ($\mu g/hr/mm^2 x10^3$).
Values represent the mean of 8 plants, 210 days post inoculation.
Bars represent 95% confidence limits.

Table 4: Leaf Water Potential at Dawn and Midday for Leaves from Inoculated and Uninoculated UF4 Clonal Palms

	Xylem Potential (MPa)		Correlation co-efficient (r)
	07.00h	14.00h	between 07.00 and 14.00h
Control	-0.237a	-0.511a	0.177*
Inoculated	-0.227a	-1.03b	0.974**

Values represent the mean of 8 plants and were taken from mid-canopy leaves 212 days post inoculation.
Within each column, values with the same letter are not significantly different (P > 0.01, Mann Whitney U-Test).
* = not significant, ** = Significant (P < 0.005)

In a subsequent experiment with a different group of UF4 clonal palms (but at the same stage of symptom development) stunted leaves had a reduced stomatal conductance and internal CO_2 and an increased photosynthetic rate, but none of these changes was statistically significant (Table 5).

Table 5: Stomatal Conductance and Rate of Photosynthesis of Inoculated and Uninoculated UF4 Clonal Palms.

	Stomatal Conductance (mMol m^{-2} s^{-1})	Photosynthesis (μMol m^{-2} s^{-1})	Internal CO2 (ppm)
Control	83.1	2.83	299.9
Inoculated	76.1	3.46	241.4

Values were obtained from mid-canopy leaves, and are the mean of 10 replicate palms 250 days post inoculation.
There are no significant differences between values (P > 0.05, Mann Whitney U- Test).

These results established that severely stunted palms were suffering from mild water stress, which was insufficient to affect the rate of transpiration and photosynthesis per unit leaf area. However, the reduced leaf area of these plants resulted in a overall decrease in transpiration and photosynthesis for each palm.

3.2.2.b. Stunted Palms with Chlorotic Leaves

Infected palms were selected and divided into 2 groups :- 1) "stunted palms" where there was little or no leaf chlorosis and the 5th leaf was stunted, and 2) "chlorotic palms" where there was extensive chlorosis and the 5th leaf was highly (>40%) chlorotic.

Measurements were made of stomatal resistance, water potential, solute potential, relative water content (RWC) and percent dry weight (dry weight divided by fresh weight) (2.3.4,5,6,7) of the 5th leaf of all palms. In addition, measurements were made of midday stomatal resistance and water potential of the 8th leaf. This leaf was chosen because even on palms with extensive chlorosis it was not chlorotic. Measurements would therefore indicate if differences between chlorotic and non-chlorotic 5th leaves reflected differences in the water relations of entire plants or merely between chlorotic and non-chlorotic leaves.

Stunted, non-chlorotic palms showed signs of greater water stress than in the previous experiment. Thus stomatal resistance of both 5th and 8th leaves was greater

than controls and dawn water potential of the 5th leaf was reduced (Tables 6 & 7). As previously, midday water potential of these non-chorotic leaves was lowered (Table 7), but with the exception of the midday water potential of the 8th leaf, none of these differences was statistically significant.

Table 6: Midday Stomatal Resistance of Uninoculated and Inoculated Chlorotic or Non-chlorotic Palms

	Stomatal Resistance (s cm^{-1})	
	5th Leaf	8th Leaf
Control	9.52a	6.72a
Non-chlorotic	15.6a	13.75a
Chlorotic	81.16b	20.66b

Values are the mean of 10 replicate UF4 clonal palms, 266 days post inoculation.
Within each column, values with the same letter are not significantly different (P > 0.05, Kruskall Wallis and repeated Mann Whitney U-Test).
Chlorotic palms had > 40% chlorosis of the 5th leaf

Observations of chlorotic palms indicated that these plants were more severely water stressed, with water potential and stomatal resistance values significantly different to controls (Tables 6 & 7). In particular the chlorotic 5th leaves had very high stomatal resistance combined with extremely low dawn water potential and therefore appeared to be suffering from permanent acute water stress.

Dawn measurements of RWC and percentage dry weight of the 5th leaf from stunted and control palms were very

similar (Table 8); but it was not possible to obtain RWC values for chlorotic leaves since no fluid was forced from the petiole at the pressure applied (2.3.6). The percentage dry weight of chlorotic leaves was higher than for stunted or control leaves, but this difference was not statistically significant. Thus, RWC and percentage dry weight provided relatively insensitive measurements of host water stress.

Table 7: Leaf Water Potential of Uninoculated and Inoculated Chlorotic or Non-chlorotic Palms

	Leaf Water Potential (MPa)		
	5th Leaf		8th Leaf
	Dawn	Midday	Midday
Control	-0.128a	-0.441a	-0.352a
Non-chlorotic	-0.235a	-0.817ab	-0.559b
Chlorotic	-1.063b	-0.998b	-0.616b

Values are the mean of 7 replicate UF4 clonal palms, 266 days post inoculation.
Within each column, values with the same letter are not significantly different ($P > 0.05$, Kruskall Wallis and repeated Mann Whitney U-Test).
Chlorotic plant had > 40% chlorosis of the 5th leaf.

Measurements of symplast solute potential at dawn indicated a significant increase in solute concentration for both stunted and chlorotic leaves (Table 9). Measurements of apoplast solute potential were much higher (*i.e.* lower solute concentration) but followed the same trend as symplast potential. However, the lower apoplast values for diseased plants may reflect the very small

volume of fluid collected from some leaves, at the pressure used (1.45MPa). At the same pressure much larger volumes of fluid were obtained from control leaves (due to their higher water potential and greater size); and the first 25μl collected from such leaves had a much lower solute potential (-0.107 MPa) than the subsequent 50 μl (-0.0146 MPa). Therefore, values of apoplast solute potential obtained by this method are probably too low, particularly for diseased plants.

Table 8: Leaf Relative Water Content and Percent Dry Weight for Uninoculated and Inoculated Chlorotic or Non - chlorotic Palms.

	Relative Water Content	Percent Dry Weight
Control	99.6	27.2
Non-chlorotic	99.5	27.2
Chlorotic	-	31.0

Values are from the 5th leaf from 7 replicate UF4 plants, 266 days post inoculation.
Within each column, there are no statistically significant differences ($P > 0.05$, Kruskall Wallis).
RWC measurements for chlorotic leaves were not performed (see Text).
Chlorotic palms had > 40% chlorosis of the 5th leaf.

Cell turgor (estimated by subtracting symplast solute potential from leaf water potential) at dawn was higher than controls in stunted leaves but reduced in chlorotic leaves (Table 9), although none of these differences were statistically significant.

In summary, leaves from stunted plants (measured 266 days post inoculation) were more water stressed than those on comparable plants measured 212 days post inoculation (see previous section) but were still able to recover at night. Palms with several chlorotic leaves were more water stressed than stunted palms and their chlorotic leaves were severely and permanently water stressed. The solute potential of diseased palms was reduced, indicating osmotic adjustment to water stress.

Table 9: Dawn Solute Potential and Turgor for Uninoculated and Inoculated Chlorotic or Non-chlorotic Palms

	Solute Potential (MPa)		Turgor (MPa) *
	Apoplast	Symplast	
Control	-0.0349a	-0.886a	0.758a
Non-chlorotic	-0.0522a	-1.232b	0.963a
Chlorotic	-0.0897a	-1.753b	0.719a

Values are from the 5th leaf from 7 replicate UF4 plants, 266 days post inoculation.
Within each column, values with the same letter are no significant differences ($P > 0.05$, Kruskall Wallis & repeated Mann Whitney U-Test).
* Calculated from Leaf water potential = turgor potential + solute potential
Chlorotic palms had > 40% chlorosis of the 5th leaf.

3.2.3. Effect of Water Stress on the Development of Inoculated and Uninoculated Palms

Mild water stress can induce reductions in canopy size in otherwise healthy plants (see Introduction to this chapter). Therefore, the correlation between stunting and mild water stress observed in inoculated palms suggested

that in this disease stunting may be caused by mild water stress. To investigate this possibility water (drought) stress was applied to inoculated and uninoculated UF4 clonal palms, and their growth was compared with relevant controls.

Water stress was applied from one month post inoculation by withholding water from plants, until the first signs of stomatal closure were observed. Plants were then rewatered to the same level as controls and the process was repeated. Stomatal closure was assessed by porometer readings of mid-canopy leaves.

Both water stress and disease significantly reduced total leaf area (16 & 21% respectively) and when combined the canopy was reduced by one third (Table 10). In diseased plants, the reduction in canopy size was caused by severe leaf stunting which was partly offset by a small but significant increase in leaf number (Table 10). Water stress of uninoculated plants caused a small decrease in leaf size (*e.g.* 2 & 5% respectively for leaves 10 & 7), however, the reduction in total leaf area was mostly because of a decrease in leaf number (Table 10).

Water stress had no significant effect on the frequency of stem browning which occurred in 93% of water stressed and 85% of non water stressed inoculated plants.

These results indicate that water stress had minimal effect on leaf size and therefore is probably not directly responsible for the leaf stunting observed in this

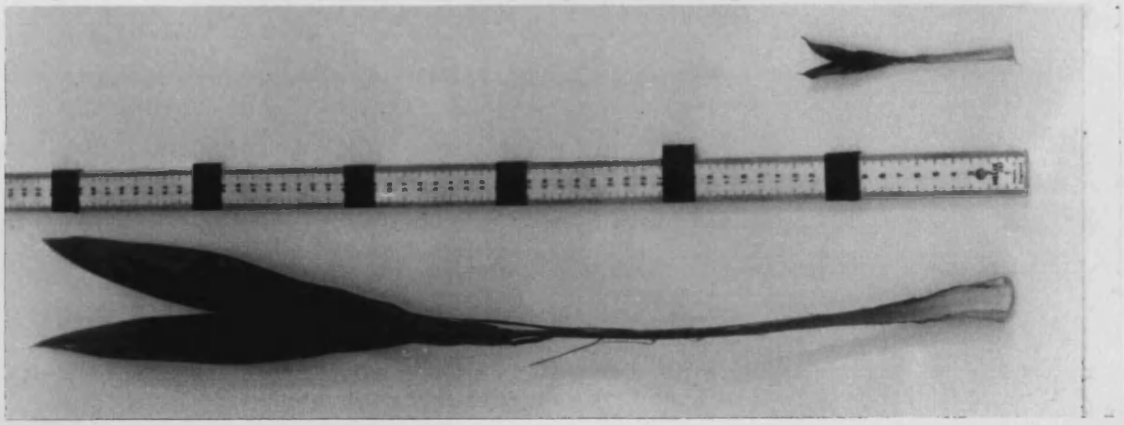
disease. Indeed, some stunted leaves from diseased plants were only 5% of the size of comparable controls (Figure 8); such severe stunting suggested a substantial alteration to the processes of plant growth.

Table 10: Effect of Disease and Water Stress on Leaf Size and Number

	Total Leaf Area ($mm^2 \times 10^3$)	Size of Leaf 10 ($mm^2 \times 10^3$)	Number of Fully Developed Leaves
Control	125.9a	18.82a	11.77ab
Control + Drought	105.2b	18.45a	11.10a
Infected	99.7b	7.18b	12.94c
Infected + Drought	84.4b	7.34b	12.42bc

Values are the mean of 20 (non-water stressed) or 14 (water stressed) replicate UF4 clonal palms. Total leaf area was measured 269 days post inoculation, leaf size and number measurements were 292 days post inoculation. Within each column, values with the same letter are not significantly different ($P > 0.05$, Kruskall Wallis and repeated Mann Whitney U-Test)

Figure 8: Severe Leaf Stunting cause by Fusarium Wilt.



The lower leaf is the youngest fully expanded leaf from an uninoculated UF4 clonal palm, the upper leaf is its equivalent from an inoculated palm (*ca.* 200 days post inoculation).

3.2.4. Effect of Disease on Cell Division, Cell Elongation and Internal Architecture of Stunted Leaves.

A reduction in leaf surface area could be caused by decreases in cell size and/or number. These possibilities were investigated by comparing leaves from severely stunted diseased and uninoculated UF4 palms. Measurements were made of leaf surface area and upper epidermal cell surface area (2.3.1.d) and from these values estimates of the number of cells per leaf epidermis were calculated.

In diseased palm leaves, there were significant reductions in the size (65% of control) and number (18% of control) of upper epidermal cells (Table 11). Therefore stunting was due to a decrease in both cell division and elongation, with reduced cell division causing most (*ca* 70%) of the effect.

Table 11: Effect of Disease on the Number and Size of Leaf Epidermal Cells.

	Leaf Area ($mm^2 \times 10^3$)	Upper Epidermal Cell Size ($mm^2 \times 10^{-3}$)	Upper Epidermal Cells per Leaf ($\times 10^6$) *
Control	18.42	0.992	18.65
Stunted	2.36	0.649	3.45

Measurements were made from 10th leaf from severely stunted or uninoculated UF4 clonal palms. Values are the mean of observations from 12 leaves (10 cells per leaf). All values for stunted leaves are significantly different to the control ($P < 0.01$, Mann Whitney U-Test).
* Calculated from leaf area ÷ cell size.

If such changes occurred throughout the leaf lamina, they could alter the thickness of the leaf, internal air

space volume and mesophyll cell size and therefore may affect the photosynthetic efficiency of the leaf. Therefore, the 3 above parameters were measured on leaf samples from stunted and control palms (2.3.1.d).

The thickness and air space volume of stunted leaves was almost identical to those of controls, and although mesophyll cell volume was reduced in stunted leaves by *ca* 20% this difference was not statistically significant (Table 12).

These results again indicated that leaf stunting was mostly due to a reduction in cell division, which surprisingly did not affect leaf thickness or air space volume.

Table 12: Effect of Disease on Leaf Internal Dimensions.

	Leaf Thickness (mm)	Mesophyll Cell Size ($\mu m^3 x 10^3$)	% Air Space Volume*
Control	0.164	45.82	33.49
Stunted	0.170	39.55	33.30

Measurements were made from 10th leaf from severely stunted or uninoculated UF4 clonal palms. Values are the mean of observations from 10 leaves (10 cells per leaf). There are no significant differences between stunted and control leaves ($P > 0.05$, Mann Whitney U-Test).
* Percentage air to leaf volume.

3.2.5. Effects of a Gibberellin Synthesis Inhibitor and Infection on Leaf Growth.

The severity of leaf stunting suggested that in diseased palms there was a change in the level or activity of growth substances. Also, stunted leaves on these plants appeared unusually dark green and contained higher levels of chlorophyll a and b (respectively 3.41 & 1.17 mg/g fresh weight) than comparable leaves from uninoculated palms (respectively 1.52 & 0.507 mg/g fresh weight) (using the method of Lichtenthaler & Wellburn, 1983).

The gibberellin synthesis inhibitor Paclobutrazol (PBZ) (Bonzi, I.C.I. Agrochemicals, Fernhurst, UK) is known to cause the production of stunted dark green leaves, and was therefore applied to uninoculated palms in order to try to simulate disease-induced stunting.

This study was conducted as 2 separate experiments on UF28 clonal palms (UF4 plants, as used previously were not available). Initially, the effect of PBZ on leaf growth was assessed, and this was then compared with the effect of disease on the same characteristics.

The dose of PBZ needed to induce stunting in uninfected oil palm was unknown and therefore initially 3 levels of PBZ were tested. The growth inhibitor was applied, as a soil drench (100ml/palm) to plants (4 weeks after repotting) at "high" (50mg a.i./plant), "medium" (5mg a.i./plant) or "low" (0.5mg a.i./plant) concentration (5mg a.i. / 100ml is the recommended rate for the stunting of tulips).

To simulate a possible increase in stimulus to stunting as infection progressed, the medium and low treated plants received a second application of PBZ at normal dose 42 days later, followed by a treatment at high dose 91 days after the first application. The plants were examined 288 days after the first application.

Leaf stunting induced by PBZ appeared very similar to that found in diseased plants (see Figure 9) and the higher the initial application concentration, the more severe was the stunting; the area of the 9th leaf from high, medium & low treated plants were respectively 27, 70 & 79% of the control. The palms initially treated with the high dose most closely resembled stunted diseased plants, and therefore leaves from these palms and comparable leaves from diseased plants (*eg.* mature and stunted to a similar degree) were examined further.

In order to identify the mechanism(s) of stunting it was necessary to demonstrate the effect of disease and PBZ on cell size and number. This was calculated from measurements of leaf area, petiole length, the size of petiole parenchyma cells, leaf upper epidermal cells and petiole xylem vessel diameter (2.3.1.b,d).

PBZ caused stunting through a large reduction in both leaf area and petiole length (respectively 28 & 21% of controls) and similar reductions in both these parameters were observed in diseased palms (Table 13a,b)

Figure 9: Paclobutrazol-Induced Stunting in UF 28 Clonal Palms.

The Plant on the left is 250 days post treatment with a high dose of PBZ, the palm in the right is 230 days post inoculation with Foe, the central palm is an untreated control.

Table 13a: Effect of Paclobutrazol(PBZ) on the Leaf Growth of UF28 Clonal Palms.

	Petiole Length (mm)	Leaf Area ($mm^2 x 10^3$)
Control	247.5	66.0
PBZ	51.6	18.6

Values are the mean of measurements of the 9th leaf from 10 palms, 280 days after treatment with PBZ. †
Within each column, all values are significantly different ($P < 0.01$, Mann Whitney U-Test).

Table 13b: Effect of Disease on the Leaf Growth of UF28 Clonal Palms

	Petiole Length (mm)	Leaf Area ($mm^2 x 10^3$)
Control	224.6	39.2
Infected	89.6	6.74

Values are the mean of measurements of the 7th leaf from 10 palms, 240 days post inoculation. †
Within each column, all values are significantly different ($P < 0.01$, Mann Whitney U-Test).

PBZ resulted in an increase in upper epidermal cell width but a reduction in cell length and surface area (respectively, 114, 76 & 86% of controls); in diseased palms epidermal cell length, width and surface area were all reduced (respectively 78, 93 & 72% of controls) (Tables 14a, b). Both PBZ and infection resulted in large reductions in the estimated number of cells per leaf epidermis (respectively 34 & 22% of controls) (Table 14a, b).

Table 14a: Effect of Paclobutrazol(PBZ) on Leaf Epidermal Cell Size and Number

	Upper Epidermal Cell Length (μm)	Width (μm)	Area ($mm^2 x10^{-3}$)	Calculated Cells Per Upper Epidermis of Leaf ($x10^6$)
Control	49.6	18.3	0.894	72.1
PBZ	37.9	20.8	0.767	24.2

Values are the mean of 10 measurements of the 9th leaf of 10 UF28 clonal palms, 280 days after the application of PBZ.
Within each column, all values are significantly different ($P < 0.01$ for cell length and cell per leaf, $P < 0.05$ for cell width and area; Mann Whitney U-Test).

Table 14b: Effect of Disease on Leaf Epidermal Cell Size and Number

	Upper Epidermal Cell Length (μm)	Width (μm)	Area ($mm^2 x10^{-3}$)	Calculated Cells Per Upper Epidermis of Leaf ($x10^6$)
Control	60.6	17.9	1.068	36.22
Infected	47.0	16.6	0.768	7.82

Values are the mean of 10 measurements of the 7th leaf of 10 UF28 clonal palms, 240 days after inoculation.
Within each column, all values are significantly different ($P < 0.01$ for all cell measurements, $P < 0.05$ for cell per leaf; Mann Whitney U-Test).

Similar results were obtained from measurements of petiole cell size (Table 15a,b). PBZ induced reductions in parenchyma cell length, width and the estimated number of cell lengths per petiole length (respectively 67, 90 and 30% of control); in infected palms these 3 parameters were also reduced (respectively 68, 76 and 54% of controls).

Table 15a: Effect of Paclobutrazol(PBZ) on Petiole Cell Size and Number

	Parenchyma Cell Length (μm)	Parenchyma Cell Width (μm)	Parenchyma Cell Lengths Per Petiole Length ($x10^3$)
Control	68.1a	51.4a	36.7a
PBZ	45.7b	46.4a	11.1b

Values are the mean of 10 measurements of the 9th leaf of 10 UF28 clonal palms, 280 days after the application of PBZ.
Within each column, values with the same letter are not significantly different ($P > 0.01$, Mann Whitney U-Test).

Table 15b: Effect of Disease on Petiole Cell Size and Number

	Parenchyma Cell Length (μm)	Parenchyma Cell Width (μm)	Parenchyma Cell Lengths Per Petiole Length ($x10^3$)
Control	70.8	48.4	31.1
Infected	48.4	37.5	16.7

Values are the mean of 10 measurements of the 7th leaf of 10 UF28 clonal palms, 240 days after inoculation.
Within each column, all values are significantly different ($P < 0.01$, Mann Whitney U-Test)

Thus, in diseased palms there were significant reductions in both cell length and width, and in PBZ treated plants cell length was reduced (Tables 14 & 15). However, cell width in PBZ treated palms was either increased (Table 14) or only marginally decreased (Table 15). Similarly, while xylem vessel diameter was halved in diseased palms, in PBZ-treated palms vessel diameter was 83% of controls (Table 16a, b).

Table 16a: Effect of Paclobutrazol(PBZ) on Xylem Size and Number and Petiole Resistance and Resistivity.

	Xylem Vessel		Petiole	
	Diameter (μm)	Number Per Petiole	Resistance Per m Length (MPa s m^{-3}) x10^6	Resistivity * (MPa s m^2 m^{-3}) x10^6
Control	29.2a	368a	37.9a	1.30a
PBZ	24.2b	311a	94.3b	0.288b

Values are the mean of measurements of the 9th leaf of 10 UF28 clonal palms, 280 days after application of PBZ.
* Resistivity = Resistance adjusted for leaf area and petiole length.
Within each column, values with the same letter are not significantly different (P > 0.01, Mann Whitney U-Test).

Table 16b: Effect of Disease on Xylem Size and Number and Petiole Resistance and Resistivity.

	Xylem Vessel		Petiole	
	Diameter (μm)	Number Per Petiole	Resistance Per m Length (MPa s m^{-3}) x10^6	Resistivity * (MPa s m^2 m^{-3}) x10^6
Control	28.8a	254a	61.3a	1.11a
Infected	14.4b	184b	1975.2b	2.42a

Values are the mean of measurements of the 7th leaf of 10 UF28 clonal palms, 240 days after inoculation.
* Resistivity = Resistance adjusted for leaf area and petiole length.
Within each column, values with the same letter are not significantly different (P > 0.05, Mann Whitney U-Test), Xylem diameter and resistance values are significantly different at P < 0.01 (Mann Whitney U-Test).

The reduction in vessel diameter could have very significant effects on vessel conductivity. Since, following the Hagen-Poiseulle law (and assuming xylem vessels function as perfect cylinders) then the flow through a vessel is proportional to the cube of the vessel diameter. Thus, a 50% reduction in vessel diameter would cause a 94% reduction in flow.

In plants treated with PBZ, a 17% reduction in xylem diameter combined with a 15% reduction in vessels per petiole resulted in an increase (x2.5) in calculated petiole hydraulic resistance (2.3.8.b.) (Table 16a). However, due to the smaller leaf area and petiole length from stem to mid lamina, the petiole resistivity of treated plants was significantly less (22%) than that of controls (Table 16a).

In diseased palms, there were larger reductions in vessel diameter and vessels per petiole (respectively 50 and 28%) which resulted in a very large (x32.2) increase in petiole resistance (Table 16b). This effect was also reduced by leaf stunting, but diseased palms maintained a higher (x2.18) petiole resistivity than controls, although this difference was not statistically significant (Table 16b).

In summary, the application of a gibberellin inhibitor mimicked the leaf stunting observed in diseased palms and, as in infected plants, this was mostly due to a reduction in cell division. However, in diseased palms, cell width

was reduced to a greater extent than in PBZ-treated palms. Due to the very large effect of xylem diameter on vessel conductivity, this resulted in a significant difference in estimates of petiole resistivity for diseased and PBZ-treated palms.

3.2.6. Effect of Gibberellin on Infected and Uninfected Palms

Since the application of a gibberellin inhibitor to healthy palms simulates disease-induced stunting then applying gibberellin to infected palms might prevent stunting. In order to test this theory, gibberellin was applied to infected and control UF4 clonal palms.

UF4 clonal palms were propagated and inoculated at the low dose described earlier (2.1 & 2.2). When symptoms were first visible, 90 days post inoculation, gibberellic acid (> 90% GA3; Sigma, Poole, Dorset) was applied as a foliar spray (to "run-off") to both inoculated and control palms. The treatment was repeated at weekly intervals thereafter until the end of the experiment. Two concentrations of GA3 were tested (10^{-4} and 10^{-5} molar) and untreated palms were sprayed with distilled water. The higher concentration of GA3 was the most effective and therefore only results with this treatment are presented.

Measurements were made of petiole length, leaf area, epidermal and parenchyma cell size, xylem vessel diameter and number of vessels per petiole. From these values it was possible (as in the previous section) to calculate the

number of upper epidermal cells per leaf, parenchyma cell lengths per petiole, and petiole hydraulic resistance and resistivity. Measurements were also made of midday leaf water potential and browning of bulb stem tissue (2.3.5 & 2.3.2.b).

In infected palms not treated with GA3 there were large reductions in petiole length and leaf area (respectively 33 and 47% of control, Table 17) which were almost entirely due to reductions in cell numbers (Tables 18 and 19), although there were also small, but usually insignificant, reductions in cell size.

The application of GA3 to uninfected palms had no significant effect on leaf area or the size and number of cells per leaf lamina (Tables 17 and 18). In contrast, GA3 induced large increases (104%) in petiole length (Table 17) which were caused by a similar (89%) increase in cell number accompanied by much smaller increases in cell size (Table 19).

When GA3 was applied to infected palms it had no effect on leaf area or the number of cells per leaf epidermis (Tables 17 and 18), it did however cause a small but significant increase in epidermal cell length.

In contrast, in petioles, GA3 and infection had opposite and almost equal effects on petiole length and parenchyma number and length (Tables 17 & 19). However, although GA3 significantly increased petiole parenchyma cell width in uninfected palms, it had no effect on

parenchyma width in infected palms which were significantly less than controls (Table 19).

Table 17: Effect of Infection and Gibberellin on Leaf Growth.

	Petiole Length (mm)	Leaf Area (mm^2 $x10^3$)
Uninfected	507b	35.2a
Uninfected + GA3	1036c	32.2a
Infected	168a	16.6b
Infected + GA3	514b	15.7b

Values are the mean of measurements of the 8th leaf from 8 UF4 clonal palms, 330 days post inoculation.
Within each column, figures with the same letter are not significantly different (P > 0.05, Kruskall Wallis and repeated Mann Whitney U-Test).

Table 18: Effect of Infection and Gibberellin on Leaf Epidermal Cell Size and Number.

	Upper Epidermal Cell Length (μm)	Width (μm)	Area ($mm^2 x10^{-3}$)	Calculated Cells Per Upper Epidermis of Leaf ($x10^6$)
Uninfected	55.8ab	18.5a	1.03a	35.2a
Uninfected + GA3	56.1ab	17.0a	0.94a	34.2a
Infected	52.6a	16.6a	0.88a	17.6b
Infected + GA3	62.0b	15.2a	0.94a	17.2b

Values are the mean of 10 measurements of the 8th leaf of 8 UF4 clonal palms, 330 days after inoculation.
Within each column, figures with the same letter are not significantly different (P > 0.05, Kruskall Wallis and repeated Mann Whitney U-Test).

Table 19: Effect of Infection and Gibberellin on Petiole Cell Size and Number.

	Parenchyma Cell Length (μm)	Width (μm)	Parenchyma Cell Lengths Per Petiole Length ($x10^3$)
Uninfected	71.0a	45.3ab	71.7b
Uninfected + GA3	79.4a	50.2b	135.3a
Infected	61.0a	40.7c	27.2c
Infected + GA3	72.9a	39.4c	71.5abc

Values are the mean of 10 measurements of the 8th leaf of 8 UF4 clonal palms, 330 days after inoculation.
Within each column, figures with the same letter are not significantly different ($P > 0.05$, Kruskall Wallis and repeated Mann Whitney U-Test).

Similarly, the application of GA3 did not reverse the 25% reduction in xylem diameter observed in infected palms (Table 20). Both GA3 and infection reduced the number of xylem vessels per petiole and when combined caused a significant 30% reduction in vessel number (Table 20)

Table 20: Effect of Infection and Gibberellin on Xylem Size and Number and Petiole Resistance and Resistivity.

	Xylem Vessel		Petiole	
	Diameter (μm)	Number Per Petiole	Resistance Per m Length (MPa s m^{-3}) x10^6	Resistivity * (MPa s m^2 m^{-3}) x10^6
Uninfected	24.7a	303a	81.7a	1.98a
Uninfected + GA3	24.9a	239ab	113.3a	4.70b
Infected	18.6a	239ab	2582.4b	1.69a
Infected + GA3	18.7a	217b	1602.3ab	12.5b

Values are the mean of 10 measurements of the 8th leaf of UF4 clonal palms, 330 days after inoculation.
* Resistivity = Resistance adjusted for leaf area and petiole length.
Within each column, figures with the same letter are not significantly different (P > 0.05, Kruskall Wallis and repeated Mann Whitney U-Test).

As found previously, reductions in vessel diameters caused very large increases in calculated petiole resistance, which in infected palms not treated with GA3 was counteracted by reductions in leaf area and petiole length to produce values of resistivity similar to those in uninfected plants (Table 20).

In contrast, the application of GA3 to both infected and uninfected palms caused significant increases in calculated values of petiole resistivity (Table 20). In uninfected plants, this was entirely due to a increase in petiole length but in infected palms, it was because a high petiole resistance (caused by narrow xylem vessels)

was not offset by a reduction in petiole length, which occurred if GA3 were not applied.

These theoretical increases in resistivity were reflected in leaf water relations. Both GA3 and infection induced significant reductions in leaf water potential and when combined, induced severe water stress (Table 21).

Table 21: Effect of Infection and Gibberellin on Leaf Water Potential and Bulb Stem Browning.

	Leaf Midday Water Potential (MPa)	% Browning of Bulb Stem Tissue
Uninfected	-0.59a	0
Uninfected +GA3	-1.08b	0
Infected	-1.08b	78.2a
Infected +GA3	-1.71c	73.1a

Values are the mean of 8 measurements of the 8th leaf or bulb of UF4 clonal plants, 330 days post inoculation. Within each column, figures with the same letter are not significantly different ($P > 0.05$, Kruskall Wallis and repeated Mann Whitney U-Test).

The application of GA3 to infected plants had no effect on the degree of stem tissue browning, which was severe in all inoculated palms (Table 21).

In summary, the application of a gibberellin synthesis inhibitor (PBZ) to uninfected palms reduced both petiole length and leaf area, while the application of GA3 to uninfected plants only increased petiole length and had no

effect on leaf area. These changes in growth were mostly due to reductions in cell division.

In diseased palms, growth changes were very similar in both cause (reduced cell division) and effect to that induced by PBZ , and petiole stunting could be completely counteracted by the application of GA3. These results provide circumstantial evidence that stunting in diseased plants is due to changes in the level and/or activity of host gibberellin.

However, in diseased palms there were also significant reductions in xylem vessel diameter that could not be counteracted or simulated by the application of PBZ or GA3, and these changes could have significant effects on xylem hydraulic resistance.

3.2.7. Hydraulic Resistivity in Diseased Palms.

In vascular wilt diseases, water stress is usually presumed to result from vascular occlusion, but in diseased oil palm there is also a reduction in xylem diameter. Therefore, two mechanisms for increased vascular resistance are possible in this disease, and it was necessary to establish their involvement in the mild and severe water stress observed in stunted and chlorotic leaves respectively .

In these experiments, hydraulic resistivity was estimated from both soil to stem and stem to leaf by 2 independent techniques.

In the first (RTVxylem), calculations were based on measurements of xylem vessel diameter and number and occlusion in the roots and petiole (2.3.8.b). These values were then compared with a second estimate (RTVpotential/flow), derived from measurements of water potential gradients and water flow (2.3.8.a). With this second method flow was either estimated by whole plant transpiration (2.3.4.a.) or from measurements of stomatal and boundary layer resistance (2.3.4.b. Appendix 2).

Two experiments, with UF4 clonal palms were performed, the first involved palms with stunted leaves and few (if any) chlorotic leaves, while in the second, infected palms with several chlorotic leaves were examined.

3.2.7.a. Hydraulic Resistivity in Stunted Diseased Palms.

As in previous experiments, infection resulted in a large reduction (50%) in total leaf area and leaf size (Table 22). There was no significant difference in transpiration rate per unit leaf area between stunted and control palms, but transpiration estimates based on the weight loss method were approximately double those calculated from stomatal and boundary layer resistance (Table 22).

No xylem occlusion was observed in any control palms, whilst in diseased plants occlusion occurred in the roots, stem and petiole base in 28.8, 51.5 and 5.2% of vessels respectively. Mean xylem vessel diameter was significantly reduced ($P < 0.01$, Mann Whitney U-Test) in diseased palms, and this effect was more pronounced in the petioles (mean

diameter 13.6μm, 63% of control) than in the roots (mean diameter 22.6μm, 79% of control).

Leaf symptoms frequently occurred in the absence of the pathogen, which in the 9 diseased palms was reisolated from the petiole base in 6 palms, from the top of the petiole (just below the lamina) in only 2 palms and never from the mid-rib halfway up the lamina.

Table 22: Leaf Area and Transpiration of Uninfected and Infected Stunted Palms

	Leaf Area (m^2 x10^{-3})		Transpiration Rate $ (m^3 m^{-2} s^{-1} x10^{-9})	
	Whole Plant	Measured Leaf *	Weight Loss	Stomatal Resistance
Control	122.8a	22.6a	22.4a	10.4a
Infected	60.8b	6.73b	26.1a	11.7a

Values are the mean of 9 replicate UF4 clonal palms, 510 days post inoculation.

$ Transpiration was estimated from whole plant weight loss or estimates of stomatal and boundary layer resistance of a mid-canopy leaf.

* The mid-canopy leaf from which stomatal resistance and leaf water potential measurements were obtained.
Within each column, values with the same letter are not significantly different (P > 0.05, Mann Whitney U-Test).

In uninoculated palms, the water potential gradient between soil and stem was much larger (x3.2) than between stem and leaf (Table 23). In diseased palms both stem and leaf water potentials were significantly reduced, and water potential gradients were increased; in particular

the stem to leaf gradient was much larger (x2.95) than in control plants (Table 23).

Table 23: Water Potentials and Water Potential Gradients in Uninfected and Infected Stunted Palms.

	Water Potential (MPa)		Water Potential Gradient (MPa)	
	Stem	Leaf	Soil/Stem	Stem/Leaf
Control	-0.251a	-0.321a	-0.236a	-0.075a
Infected	-0.333b	-0.553b	-0.318b	-0.221b

Values are the mean of 9 replicate UF4 clonal palms, 510 days post inoculation.
With each column, values with the same letter are not significantly different ($P > 0.05$, Mann Whitney U-Test).

In infected palms, there were increases in calculated values of resistivity, between the soil and stem (Table 24), RTVxylem estimates (which ignore pre vascular resistances) indicated a 90% increase in vascular resistivity. In contrast, values of RTVpotential/flow (which were much higher, *ca.* x100, than RTVxylem for both control and inoculated plants), indicated a 20 to 40% increase in resistivity in this region.

Table 24: Calculated Hydraulic Resistivity from Soil to Stem for Uninoculated and Inoculated Stunted Palms.

	Resistivity (MPa s m^2 m^{-3} $x10^6$)		
	Xylem *	Flow/Potential $	
		Method 1 (Weight Loss)	Method 2 (Porometer)
Control	0.184a	11.05a	23.89a
Inoculated	0.349b	15.32a	29.08a
Stunted Control	1.90	1.39	1.22

Resistivity values are the mean of 8 replicate palms measured 510 days post inoculation.
Within each column, values with the same letter are not significantly different (P > 0.05, Mann Whitney U-Test).

* Resistivity calculated from measurements of xylem diameter and blockage for 5 vascular bundles from 5 roots per plant, and measurements of leaf area and root length.

$ Resistivity calculated from the difference between soil and stem water potential ÷ transpiration rate per unit leaf area. Transpiration rate was estimated from measurements of whole plant water loss and leaf area (method 1), or calculations of stomatal and boundary layer resistance of the measured mid-canopy leaf (method 2).

From stem to leaf, estimates of RTVxylem indicated a 60% increase in vascular resistivity in diseased plants, whilst RTVpotential/flow values suggested a larger increase (*ca.* 200%) in this region (Table 25).

Table 25: Calculated Hydraulic Resistivity from Stem to Leaf for Uninoculated and Inoculated Stunted Palms.

	Resistivity (MPa s m^2 m^{-3} $x10^6$)		
	Xylem *	Flow/Potential $	
		Method 1 (Weight Loss)	Method 2 (Porometer)
Control	1.83a	3.30a	6.72a
Inoculated	2.93a	10.85b	20.67b
Stunted Control	1.60	3.29	3.08

Resistivity values are the mean of 8 replicate UF4 palms measured 510 days post inoculation.
Within each column, values with the same letter are not significantly different ($P > 0.05$, Mann Whitney U-Test).

* Resistivity calculated from measurements of xylem diameter and blockage for 10 vascular bundles from each petiole, and measurements of leaf area and distance from petiole base to mid lamina.

$ Resistivity calculated from the difference between stem and leaf water potential ÷ transpiration rate per unit leaf area. Transpiration rate was estimated from measurements of whole plant water loss and leaf area (method 1), or calculations of stomatal and boundary layer resistance of the measured mid-canopy leaf (method 2).

3.2.7.b. Hydraulic Resistivity in Chlorotic Diseased Palms.

In palms with one or more chlorotic mid canopy leaves there was a 32% reduction in overall leaf area, but chlorotic leaves were only slightly smaller (*ca.* 10%) than those on uninfected palms (Table 26). Whole plant transpiration per unit leaf area was significantly reduced (*ca.* 50%) in diseased plants, and chlorotic leaves on these palms had very low transpiration rates (*ca.* 9% of controls) (Table 26). Due to this abnormally low

transpiration rate, whole plant transpiration (and hence soil to stem resistivity) could not be calculated from porometer readings of chlorotic leaves. Conversely, calculations of stem to chlorotic leaf resistivity could not be based on estimates of whole plant transpiration.

Table 26: Leaf Area and Transpiration of Uninfected and Infected Chlorotic Palms

	Leaf Area (m^2 x10^{-3})		Transpiration Rate \$ (m^3 m^{-2} s^{-1} x10^{-9})	
	Whole Plant	Measured Leaf *	Weight Loss	Stomatal Resistance
Control	168.4a	18.15a	13.63a	17.2a
Infected	115.4a	16.01a	7.01b	1.37b

Values are the mean of 5 replicate UF4 clonal palms, 570 days post inoculation.
\$ Transpiration was estimated from whole plant weight loss or stomatal conductance.
* The mid-canopy leaf from which stomatal conductance and leaf water potential measurements were obtained
Within each column, values with the same letter are not significantly different ($P > 0.05$, Mann Whitney U-Test).

There was no xylem occlusion in any control plants but in infected palms occlusion in the roots, stem and petiole occurred in 33.4, 52.4 and 7.36% of vessels respectively. In diseased palms, xylem diameter was significantly reduced ($P < 0.05$, Mann Whitney U-Test) by 14% in both the petiole (mean diameter 20μm) and the roots (mean diameter 22.7μm). In these palms there were significant reductions in the stem and leaf water potentials and the potential gradient from stem to leaf was 2.7 times greater than in control palms (Table 27).

Table 27: Water Potentials and Water Potential Gradients in Uninfected and Infected Chlorotic Palms.

	Water Potential (MPa)		Water Potential Gradient (MPa)	
	Stem	Leaf	Soil/Stem	Stem/Leaf
Control	-0.492a	-0.814a	-0.477a	-0.321a
Infected	-0.697a	-1.576b	-0.682a	-0.879b

Values are the mean of 5 replicate UF4 clonal palms, 570 days post inoculation.
Within each column, values with the same letter are not significantly different ($P > 0.05$, Mann Whitney U-Test).

As in the previous section, leaf symptoms frequently occurred in the absence of the pathogen, which was reisolated from the petiole base of all 5 diseased palms, from the top of the petiole from only 2 leaves and never from the mid-rid half way up the lamina.

In diseased palms there were increases in calculated values of resistivity. Between the soil and stem RTVxylem estimates were 80% greater than controls, whilst RTVpotential/flow values indicated a 216% increase in resistivity in this region (Table 28). Estimates of stem to chlorotic leaf resistivity (Table 29) indicated a 46% increase in RTVxylem, but in particular a substantial (3,937%) increase in RTVpotential/flow.

Table 28: Calculated Hydraulic Resistivity from Soil to Stem for Uninoculated and Inoculated Chlorotic Palms.

	Resistivity (MPa s m^2 m^{-3} x10^6)	
	Xylem *	Flow/Potential $ (Weight Loss)
Control	0.330a	36.16a
Inoculated	0.602b	114.3b
Chlorotic Control	1.82	3.16

Resistivity values are the mean of 5 replicate palms measured 570 days post inoculation.
Within each column, values with the same letter are not significantly different (P > 0.05, Mann Whitney U-Test).

* Resistivity calculated from measurements of xylem diameter and blockage for 5 vascular bundles from 5 roots per plant, and measurements of leaf area and root length.

$ Resistivity calculated from the difference between soil and stem water potential ÷ transpiration rate per unit leaf area. Transpiration rate was estimated from measurements of whole plant water loss and leaf area

Table 29: Calculated Hydraulic Resistivity from Stem to Leaf for Uninoculated and Inoculated Chlorotic Palms.

	Resistivity (MPa s m^2 m^{-3} x10^6)	
	Xylem *	Flow/Potential $ (Porometer)
Control	1.46a	19.8a
Inoculated	2.13a	799.3b
Chlorotic Control	1.46	40.37

Resistivity values are the mean of 5 replicate UF4 palms measured 570 days post inoculation.
Within each column, values with the same letter are not significantly different (P > 0.05, Mann Whitney U-Test).

* Resistivity calculated from measurements of xylem diameter and blockage for 10 vascular bundles from each petiole, and measurements of leaf area and distance from petiole base to mid lamina.

$ Resistivity calculated from the difference between stem and leaf water potential ÷ transpiration rate per unit leaf area. Transpiration rate was obtained from estimates of stomatal conductance and boundary layer resistance of the measured leaf.

In summary, stunted diseased palms were mildly water stressed and had significant reductions in leaf area and xylem diameter in the roots and petiole. These plants had appreciable levels of vascular occlusion in the roots and stems but not in the petiole base. Chlorotic leaves on diseased palms were severely water stressed, and reductions in leaf size and xylem diameter were less than in diseased stunted palms. However, the level of vascular occlusion in palms with chlorotic leaves was very similar to that observed in stunted diseased palms.

Absolute values of resistivity varied depending on the method of calculation, environmental conditions and the age of the plants. These inconsistencies could be partly overcome by using the data to compare the ratio of diseased to control resistivities, but direct comparisons are still difficult. However, the results do indicate that the severe water stress of chlorotic leaves was due to a massive increase in stem to leaf resistivity, and this could not be explained by the insignificant increase in resistivity caused by reductions in xylem diameter and xylem occlusion in the petiole.

3.2.8. Symptom Development and Physiology of UF4 Clonal Field Palms

During a visit to a oil palm plantation in Zaire (November 1989) an increment borer was used to quantify vascular occlusion in the trunk of 13 six year old UF4 clonal palms (2.3.2.c), and 3 months later (in the middle of the dry season) Dr Gail Smith (Unilever Plantations Group) measured the number of leaves, area of the 17th leaf, stomatal conductance and photosynthesis of the palms (2.3.1.b & 2.3.4.c). Stomatal conductance and photosynthesis measurements were taken from 3 replicate leaflets on leaves 5,9 and 17 (leaf 1 is the youngest fully expanded leaf) of each palm.

The palms were in 2 replicate blocks, which were sampled on alternate days, for stomatal conductance and photosynthesis. Each palm took approximately half an hour to sample and would be assessed 2 or 3 times during the

sampling period (from 07.00 to 12.00h). On some days the relative humidity (RH) remained high throughout the sampling period, while on other days a rapid fall in RH was observed. To reduce the effect of these environmental factors, the data for all palms on 2 days of relatively low vapour pressure deficit (v.p.d.) (mean midday air RH 58%, 33^{o}C) and 2 days of high v.p.d. (mean midday air RH 30%, 40^{o}C) were analysed separately.

Photosynthesis and conductance values generally declined during the sampling period and therefore, because palms were sampled at different times, direct comparison of these data were not possible . To reduce this effect, a linear regression analysis for the data over time was performed, and values for each palm were converted to a percentage of the expected value at that time. The mean of these percentages was then plotted against estimates of trunk vessel blockage (obtained from examination of trunk core samples) for each palm (Table 30).

In diseased palms (*ie*. with brown vascular bundles in the stem) there were significant reductions in the size and number of leaves (Table 30), and both these characteristics were negatively correlated with the percentage of brown vascular bundles in trunk core samples (leaf size $r=-0.72$, $P < 0.01$; number of leaves $r=-0.63$, $P < 0.05$). Leaf number was presumed to be reduced in diseased plants due to the premature senescence of older leaves (frequently observed in diseased palms) which are removed following standard plantation practice.

Table 30: Effect of Disease on Leaf Size and Number in Six Year Old UF4 Clonal Field Palms.

	Percentage Blockage of Stem Vascular Bundles *	Area of Leaf 17 (m^2)	Total Number of Green Leaves
Control	0	7.14a	47.3a
Infected	42.6	4.37b	33.4b

Values are the mean of 3 uninfected palms (with no brown vascular bundles in core stem samples) and 9 infected palms.
* From examination of 4 stem core samples per palm, taken 500mm above ground level.
Within each column, values with the same letter are not significantly different (P > 0.05, Mann Whitney U-Test)

Stomatal conductance declined during the day, and this effect was more pronounced on days of high v.p.d. (*ca* 30mMol m^{-2} s^{-1} at 07.00h to 5mMol m^{-2} s^{-1} at 12.00h), there was no correlation between the percentage of brown vascular bundles in the stem and the percentage of expected stomatal conductance ($r=0.14$, $P > 0.05$).

On days of relatively low v.p.d., net photosynthesis did not decrease with time and therefore reduced stomatal conductance on these days may have been counter balanced by an increased leaf temperature (G.Smith, 1990, pers comm). As with stomatal conductance, there was no correlation between the percentage of expected photosynthesis and the percentage of brown vascular bundles in the stem .

In contrast, on days of relatively high v.p.d., photosynthesis decreased significantly over time ($r=-0.63$, $P < 0.01$), which may reflect more complete stomatal

closure on these days. There was also a correlation ($r=-0.38$), although not quite statistically significant, between xylem blockage and the percentage of expected photosynthesis, which suggested that disease may cause reductions in photosynthesis under extreme conditions.

Thus, symptoms in UF4 clonal field palms were similar at both field and seedling/plantlet stage *ie*. reduced leaf size, premature senescence of older leaves, but no significant reduction in the rate of transpiration or photosynthesis per unit leaf area.

3.3.DISCUSSION

This is the first report of the effects of infection by Foe on the physiology and development of oil palm and, as in other vascular wilt diseases, infection resulted in host water stress. In young artificially inoculated clonal palms, two types of symptom development and water stress were observed. In the first, palms became stunted due to a reduction in the size of new leaves and although the water and osmotic potentials of these leaves were lower there was no reduction in stomatal conductance, transpiration or photosynthesis per unit leaf area. These palms therefore appeared to be suffering from prolonged mild water stress and the adjustments in osmotic potential and canopy size were comparable with responses observed in other plant species to moderate water stress (Bradford & Hsiao, 1982; Morgan, 1984), and in vascular wilt diseases where severe host water stress did not occur (Threlfall, 1959; Harrison, 1971; Tzeng *et al.*, 1985; Goodwin *et al.*, 1988b; Dey & Van Alfen, 1979).

In the second type of symptom development, older leaves of more severely diseased palms were chlorotic and severely water stressed with large reductions in leaf water and solute potential, stomatal conductance and rates of transpiration and photosynthesis. Such symptoms have frequently been reported in other studies of vascular wilt diseases (Dey & Van Alfen, 1979; Goodwin *et al.*, 1988a, Duniway, 1971b; Harrison, 1970; 1971) .

Similarly, limited observations of naturally infected field palms of the same clone, during the dry season, demonstrated that disease resulted in a reduction in leaf size and number of leaves (presumably due to premature senescence of older leaves). There was no significant reduction in the transpiration or photosynthetic rate per unit leaf area, but results indicated that under conditions of severe vapour pressure deficit photosynthesis may be reduced in diseased palms.

Thus, diseased field palms behaved in a similar manner to inoculated nursery palms. However, these observations should be repeated during the rainy season when low soil water potential and a high vapour pressure deficit are not limiting transpiration rates (G.Smith, pers comm, 1991) and the effects of vascular occlusion in diseased palms may be more apparent. Even then, disease induced water stress may not be apparent, because mature field palms (with their large stem and root system) are regarded as more drought resistant than young palms (Villalabus, Umana & Chinchilla, 1992).

However, disease induced reductions in canopy size would reduce yield without any decrease in photosynthetic rate per unit leaf area. Furthermore, observations of vascular symptoms in the stems of field palms (Renard & de Franqueville, 1989; Mepsted, Nyanduza, Flood & Cooper, 1991; and Chapter 4, this study) indicate that in some crosses 50% of palms, with no obvious external symptoms, were infected, and Renard and de Franqueville (1989)

reported a 3% to 15% yield reduction in such palms. The time scale of disease progress in these palms and mechanism of yield reduction are unknown and require further investigation.

In some diseased seedlings, values of stomatal conductance and photosynthesis were decreased whilst calculated internal CO_2 levels (CI) increased. This suggested that a non-stomatal reduction in photosynthesis had occurred, and similar results were reported in droughted (non infected) oil palms by Corley (1976) and Potulski, Barrett & Smith (1988). In contrast, in infected clonal palms a small decrease in stomatal conductance corresponded to a decreased CI and increased photosynthesis.

However, interpretation of I.R.G.A. data in this manner may be misleading due to non-uniform stomatal closure which can lead to over estimates of CI (Downton, Loveys & Grant, 1988; Terashima, Wong, Osmond & Farquhar, 1988). Further observations would be necessary to determine if the decrease in photosynthesis in this disease was entirely due to stomatal closure, and if it were more than could be expected in similarly water stressed uninfected plants.

In *Verticillium* wilt of potato, Bowden & Rouse (1991a,b) and Haverkort, Rouse & Turkensteen (1990) reported that reduction in photosynthesis and increases in CI were due to water stress. However, Hampton,Wullschleger

& Oosterhuis (1990) suggested that non-stomatal reductions in photosynthesis in *Verticillium* wilt of cotton were not due to water stress. In part this result may reflect the difficulty of simulating disease induced water stress in uninfected plants.

In vascular wilt diseases, large increases in xylem resistance (Duniway, 1971b; Street & Cooper, 1984) are generally attributed to vessel blockage (Beckman, 1964; Phipps & Stipes, 1976; Robb *et al.*, 1981). However, in this disease increased resistance may also be caused by the significant reductions in xylem diameter observed in stunted palms. These observation contradict an earlier report of increased xylem diameter in the roots of diseased palms (Obuekwe & Osagie, 1989). A similar reduction in xylem diameter was reported in *Verticillium* wilt of alfalfa by Pennypacker & Leath (1986) who also suggested that this could result in increased vascular resistance.

In this study, the full implications of reduced vessel diameter were quantified by measuring xylem diameter and application of the Hagen-Poiseulle law, whereby a 50% reduction in diameter results in a x16 increase in resistance. Values obtained by this method were then compared with calculations of resistivity obtained by observation of water potential and flow.

Both methods of measuring resistivity suffer from inaccuracies. Estimates based on the Hagen-Poiseulle law

presume vessels are perfect capillaries with smooth walls and no perforation plates. Generally, as in this study, such estimates are between 50 and 5% of values calculated from measurement of flow and pressure gradient (Zimmermann & Brown, 1971; Giordano, Salleo, Salleo & Wanderlingh, 1978; Gibson, Calkin & Nobel, 1984). However, calculation of transpiration from porometer measurements (thus resistivity estimates based on these values) can be inaccurate due to difficulties in calibration, estimating boundary layer resistance and measuring leaf temperature (Idso, Allen, Kimball & Choudhury, 1989; Pearcy, Schulze & Zimmermann, 1989, Smith, per comm, 1991). Therefore, where possible transpiration was measured by whole plant weight loss.

Perhaps not surprisingly, values of resistivity varied depending on the method of estimation, environmental conditions and the age of the plants, and consequently data were used as comparative rather than actual values. Nonetheless, if estimates of petiole resistivity in uninfected palms were converted to units of "leaf specific conductivity" (m^2 MPa^{-1} s^{-1}), the values obtained (2.5 to 17 $x10^{-8}$) were comparable to those reported by Veres (1990) in Blechnum ferns (2.3 to 27 $X10^{-8}$) and by Gibson *et al.*, (1984) in the fern *Cyrtomium falcatum* (2 to 3 x 10^{-8}).

In stunted diseased palms, the effects of smaller xylem diameters and vessel blockage were largely compensated for by reductions in leaf area and petiole length and the

comparatively small increases in estimated resistivity were compatible with previous observations of mild water stress in these plants.

The very severe water stress observed in chlorotic leaves on diseased palms corresponded to a massive increase in stem to leaf resistivity, as calculated from water potential and flow measurements. However in these palms there was no significant change in vessel diameter or leaf area and the extent of vascular blockage was almost identical to that observed in stunted palm. Petiole resistivity calculated from these parameters was also similar to that for stunted plants. Therefore the cause of this large increase in petiole resistivity was not immediately apparent.

It is possible that some resistivity values were over estimated due to inaccuracies in porometer readings. Thus, porometer readings of stomatal conductance in this study (*ca.* 50 mMol m^{-2} s^{-1}) were lower than previous reports for palms of a similar age (*ca.* 80-180 mMol m^{-2} s^{-1}) (Smith, 1989; Henson, 1991). Furthermore, Potulski, Smith & Barrett (1988) estimated minimal non-stomatal leaf conductance to be *ca* 7 mMol m^{-2} s^{-1}, while in these experiments, an estimate of *ca.* 1.5 mMol m^{-2} s^{-1} was obtained. The porometer therefore appeared to underestimate stomatal conductance and this may be more pronounced in measurements at very low transpiration rates. Misho & Yokoi (1991) reported similar inaccuracies in porometer readings at low transpiration rates and gave

a similar explanation of increased calculated flow resistance in a range of plant species at dusk. However, in this study the calculated increase in non-stunted, chlorotic leaf resistivity was much higher (x40) than observed by Mishio & Yokoi (x3) and the low dawn water potentials of these leaves indicated that these estimates were probably correct.

Alternatively, vascular blockage in the petioles of these leaves may have been underestimated since only symptoms visible with a light microscope (in particular browning of xylem parenchyma) were recognised. In a survey of wilts caused by *Verticillium* species, Robb *et al.*, (1981) concluded that the degree of leaf flaccidity was inversely correlated to vascular browning, but positively correlated with the proportion of lipid-coated vessels. Such lipid-coating would not have been noticed in this study and plugging of pit membranes by macromolecules (such as fungal polysaccarides), as suggested by Van Alfen *et al.*, (1987) would also not be detected. However, since the frequency of xylem browning and reisolation of the pathogen from the petiole were similar for both stunted and chlorotic leaves it seems unlikely that such invisible blockages were only present in chlorotic leaves. Furthermore chlorosis, which was always associated with severe water stress, only occured in non-stunted leaves, while stunted leaves on infected palms were always green, and therefore presumably not severely water stressed.

Another possible form of invisible vessel blockage are air embolisms which occur when microbubbles of air are drawn through pit membrane pores into xylem vessels (Sperry & Tyree, 1988). These embolisms would disappear as soon as xylem tension was released, e.g. when the petiole was cut for sectioning (Zimmermann, 1983), but they have been detected in Dutch Elm disease (Newbanks *et al.*, 1983). Furthermore, as suggested by Zimmermann (1983) and confirmed by Tyree, Dixon, Tyree & Johnson (1984), Salleo & Lo Gullo (1986) and Lo Gullo & Salleo (1991) wider vessels are more vulnerable to embolism and may be less able to recover once it occurs (Tyree & Yang, 1990). Therefore, in water stressed diseased palms, the relatively wide xylem vessels in chlorotic leaves could be more vulnerable to embolism than the narrow vessels in stunted leaves.

However, in the palm *Rhapis excelsa* embolism in the petiole did not occur until the water potential was < -2.9 MPa (Sperry, 1985, 1986) and similar results have been reported in other species (Tyree & Sperry, 1988; Tyree & Ewers, 1991). While in this study water potential was much higher in chlorotic leaves (*ca* -1.6 MPa) and therefore, unless oil palm is particularly vulnerable, significant levels of vascular embolism seem unlikely. Alternatively, xylem vessels in diseased plants, containing pathogen produced toxins or enzymes, may be more vulnerable to embolism than normal. Thus, millimolar concentrations of oxalic acid plus calcium greatly increased the

vulnerability of xylem vessels in *Acer saccharum* to embolism (Sperry & Tyree, 1988), as did volatile terpenes found in the xylem of *Pinus thunbergii* infected with the nematode *Bursaphelenchus xylophilus* (Kuroda, 1991). These chemicals were thought to act by reducing xylem fluid surface tension (Kuroda, 1991) or effectively enlarging pit pore size by increasing pit membrane elasticity (Sperry & Tyree, 1988); both these effects would make embolisms occur at higher xylem pressures (Zimmermann, 1983). Pathogen produced cell wall degrading enzymes may have the same effect by increasing pit pore size (Van Alfen, 1989). *F. oxysporum* ff.spp. produce large quantities of pectic hydrolases and lyases (Cooper & Wood, 1980), and Foe has also been shown to produce such enzymes (Trique, 1971; Obuekwe & Osagie, 1989).

The occurrence of embolisms in the petioles of chlorotic, stunted and control (from uninfected palms) leaves could be demonstrated by a variety of techniques (Lo Gullo & Salleo, 1991). Furthermore the relative susceptibility of these petioles to embolism could be established by comparing vulnerability curves (% loss of hydraulic conductance versus petiole water potential) (Sperry, 1986; Sperry, Donnelly & Tyree, 1988; Lo Gullo & Salleo, 1991); such measurements would demonstrate the susceptibility to and frequency of embolisms in diseased plants.

If narrow vessels in stunted leaves are more resistant to embolism, then the production of these leaves

represents a two fold adaptation to host water stress. Firstly, smaller leaves with shorter petioles require less water and have less vascular resistance and secondly narrow vessels reduce the risk of embolism in diseased palms. Leaves formed before infection has become established would have none of these adaptations and would therefore experience severe water stress, as observed in this study. In field palms, failure to adapt in this manner may result in the acute form of the disease, while if the host is able or has time to adapt the chronic form of the disease may occur (Corley, 1992, pers comm).

Alternatively, the susceptibility of non-stunted leaves may be because they are older and, as with older xylem vessels in *Populus tremuloides*, are susceptible to embolism (Sperry, Perry & Sullivan, 1991). However, in this study, infected stunted palms were still growing > 1.5 years after inoculation and old severely stunted leaves on these palms did not become chlorotic.

Leaf area reduction in response to drought has been reported in many plant species and is regarded as an important adaptation to drought (Grier & Running, 1977; Bradford & Hsiao, 1982). Presumably therefore stunting in diseased oil palm represents a response to water stress. However, there is an important difference between drought and disease induced water stress. In the former, stress is caused by a reduction in soil water potential, leading to reductions in root and leaf water potential, and host responses may involve root to shoot chemical signals

(Kramer, 1988; Passioura, 1988). In diseased plants, vessel blockage could cause leaf water stress without reduction in root water potential and the resultant chemical signals. Therefore, although differences in response could be expected, in oil palm these differences were large. Thus, drought-induced reductions in canopy size at the nursery stage (in this study) and in the plantation (Ochs & Daniel, 1976) were primarily caused by a decrease in leaf number rather than leaf size and the very severe stunting observed in diseased nursery and field palms was far more than could be expected as a host response to water stress (Hedden, pers comm 1991).

Drought has been shown to decrease xylem diameter in *Pinus resinosa* (Larson, 1963), and stimulate a premature transition to late wood formation (with narrow xylem vessels) in several tree species (Creber & Chaloner, 1984). However, in *Fraxinus pennsylvanica*, although drought induced reductions in leaf area and xylem number, it had no effect on xylem diameter (Shumway, Steiner & Abrams, 1991). Unfortunately, the effect of drought on xylem diameter in oil palm was not measured, and therefore the role of water stress in the production of narrow vessels in this disease remains unclear.

The magnitude of the reductions in leaf area in diseased palms, which is far greater than observed in uninfected plants in response to water stress, suggests that the mechanism for this change may come from the pathogen rather than the host.

Reductions in plant size have frequently been reported in vascular wilt diseases and in *Verticillium* wilt of potato, leaf stunting was mostly due to a reduction in cell size, although there was also a significant decrease in the number of cells in the leaf (Harrison & Isaac, 1968; Harrison, 1971). However, the author knows of no other report where the immediate mechanism(s) of stunting has been investigated.

Stunting in *Verticillium* wilt of tomato was attributed to increases in host IAA levels (Pegg & Selman, 1959), although generally reduced growth has, to varying degrees, been attributed to host water stress (Threlfall, 1959; Dey & Van Alfen, 1979; Goodwin *et al.*, 1988a,b; Haverkort *et al.*, 1991). However, as all plants with vascular wilt disease can be expected to suffer from water stress, to which stunting can frequently be correlated, this does not necessarily prove cause and effect. Furthermore, many experiments involve artificial inoculation of a susceptible host resulting in quick and severe water stress which could prevent the full expression of growth responses.

Fusarium wilt of oil palm provides a good model for the investigation of these effects since infected plants are mildly water stressed and can continue to grow for many months. In diseased palms, leaf stunting was mostly due to a reduction in cell division, although cell size was also reduced. The application of the gibberellin synthesis inhibitor Paclobutrazol (PBZ) to uninfected

palms produced very similar symptoms by the same mechanism while conversely, GA_3 applied to diseased palms counteracted petiole stunting through an increase in cell division. These results provide indirect evidence for stunting being caused by a reduction of host gibberellin levels. However not all growth symptoms of this disease could be simulated or counteracted by such methods. Thus, compared to stunting in diseased palms, PBZ had a relatively greater effect on the petiole than on the lamina and caused smaller reductions in xylem diameter. Furthermore, GA_3 had no effect on lamina area or xylem diameter.

Such observations do not necessarily indicate that reductions in gibberellins do not occur in diseased plants since similar discrepancies have also been observed in comparative studies of PBZ and GAs. Thus, although these compounds were generally counteractive, GA_3 could not neutralize PBZ-induced fruit stunting in apples (Prive, Elfling & Proctor, 1989) and while GA_3 influenced peach tree dry weight and root to shoot ratio, PBZ had no effect on these characteristics (Casper & Taylor, 1989). Such differences may reflect the relative sensitivity of different organs, the difficulty of selecting the correct dose and timing of application of these chemicals, and of which gibberellin, or mixture of gibberellins, to use. Furthermore, as well as reducing GA production (by inhibiting ent-kaurene oxidase) PBZ can also reduce plant growth through inhibition of sterol biosynthesis (Burden,

Carter, Clark, Cooke, Croker, Deas, Hedden, James, Lenton , 1987).

In addition to stunting effects, there were other similarities between the responses of oil palm to PBZ and to disease. At low concentrations PBZ has been reported to increase plant growth (Aloni & Pashkar, 1987) and similar responses were sometimes observed in the early stages of symptom development in this disease (Mepsted, unpublished). Additionally, in oil palm *Fusarium* wilt resulted in increases in chlorophyll content and, although chlorophyll levels were not measured, PBZ treated palms produced darker leaves and PBZ can increase chlorophyll levels in other species (Aloni & Pashkar, 1987; Smith, Roberts & Mottley, 1990).

On balance, the similar effects of PBZ and disease suggest that a growth inhibitory substance, with similar activity or activities to PBZ is produced in diseased palms. However, such effects should not be viewed in isolation and in diseased palms, growth inhibition would be combined with prolonged mild water stress, which can have profound effects on plant physiology.

Reductions in host gibberellin levels were also suggested as the reason for stunting in anther smut of campion (*Silene vulgaris*) caused by *Ustilago violacea* (Evans & Wilson, 1971). In that disease stunting could be reversed by the application of GA3 and infected plants contained lower levels of gibberellin-like substances.

Significantly, the authors also suggested that the proximity of the pathogen to the growing point of the host may be correlated with stunting, and in *Fusarium* wilt of young oil palm the pathogen is invariably present in the bulb stem tissue, which is adjacent to the growing point.

As described earlier, disease-induced stunting could be regarded as an adaptation to host water stress and it is therefore perhaps not surprising that PBZ has been shown to reduce plant susceptibility to water stress. These adaptations to drought have been related to reductions in leaf area and increases in root to leaf area (Wieland & Wamble, 1985; Aloni & Pashkar, 1987; Smith *et al.*, 1990; Smith, Gribaudo & Roberts, 1992). However, PBZ has also been shown to reduce stomatal size (Smith *et al.*, 1990. 1992) and prevent the accumulation of ABA (Wang, Sun, Zuo & Faust, 1987) and ethylene (Wang & Steffens, 1985) in apple leaves in response to water stress.

Additionally, PBZ was found to retard symptom development in *Fusarium* wilt of melon (Cohen, Yarden, Katan, Riov & Lisker, 1987). In this case, although the chemical was shown to be fungitoxic, it did not decrease host colonisation and the reduction in symptom development was ascribed to unknown changes in host metabolism.

Although these experiments have provided circumstantial evidence for reductions in host GA levels as the cause of stunting, this theory can only be fully supported by direct measurement of plant GA levels. If a GA synthesis

inhibitor is produced by the pathogen it may be obtainable from culture filtrates and the effect of these filtrates on growth and water stress tolerance of oil palm and other plant species could be tested.

4.0. SELECTION AND BREEDING OF DISEASE RESISTANT OIL PALM

4.1. INTRODUCTION.

The soil borne nature of Foe, combined with the scale of palm plantations has made control of this disease through the use of fungicides impracticable, and therefore most research effort has been directed towards the selection and breeding of disease resistant varieties.

Wardlaw (1946c, 1950) observed large differences in the incidence of wilt in adjacent blocks of field palms, and suggested that genetically controlled resistance to wilt could be expected to exist. He proposed that the then current breeding programme should be expanded to include screening for wilt resistance and as seedlings were also susceptible to the disease, they should be used to shorten the time required to the selection of resistant varieties. These ideas have remained as the principles for the selection of resistant material.

Thus, palms have been selected on the basis of field trials and nursery tests, in which young plants were artificially inoculated with Foe. Due to the comparatively short time and lack of space required, nursery screening has become the principal selection method, and is used for the assessment of > 600 crosses per annum in the Ivory Coast (de Franqueville & Renard, 1990).

Over the decades, the methods used for conducting these trials have varied considerably. Prendergast (1963) was responsible for much of the early development of

techniques for inoculum production, inoculation and disease assessment. His method involved pouring inoculum over the roots of seedlings at the 4 to 5 leaf stage, when the plants were transplanted from the prenursery to the nursery. However, he found that inoculation of younger plants by this method was less reliable due their susceptibility to transplant shock, and that root dip inoculation was also too severe. He noted that there were no immune progenies and ranked crosses with a "wilt index" by comparing the percentage of diseased or stunted palms for each cross with the mean for the whole trial. The importance of timing of disease assessment was also emphasised; observations made too early, before susceptible plants have symptoms, or too late, when relatively resistant plants have succumbed, greatly reduced the efficiency of the test.

Renard, Gascon & Bachy (1972) modified Prendergast's test by exposing the roots of the seedling, pouring inoculum onto the roots, then recovering with soil. This method caused less root damage and avoided transplant shock. Disease was assessed at the end of each trial on the basis of leaf symptoms and browning of stem tissue inside the swollen stem base (bulb). This latter technique was regarded as a more reliable indicator of disease but required destructive sampling. This group of workers also developed the prenursery wilt test in which plants were inoculated at the 1 to 1.5 leaf stage, and suggested that

this technique could reduce the length of a trial from 32 to 18 weeks.

The introduction, by Locke & Colhoun (1974), of inoculum of known concentration was an important improvement, and their use of uninoculated control plants allowed for the comparison of progenies by non-destructive growth measurements. They observed variation in symptom development caused by different fungal isolates and that an increase in the quantity of inoculum produced more severe symptoms. Subsequent workers (Meunier, Renard & Quillec, 1979; de Franqueville, 1984; Ho, Varghese & Taylor, 1985a,b; Flood, Cooper & Lees, 1989) adopted the use of a standardised inoculum concentration, but found liquid inoculum to be more practicable than the infested soil method of Locke & Colhoun (1974).

None of the methods involved the deliberate damage to roots, which could increase disease severity (Prendergast, 1963; Renard, 1970). However, when inoculation was combined with replanting or uncovering of roots (Fraselle, 1951; Prendergast, 1963; de Franqueville, 1984; Ho *et al.*, 1985a) or injection of inoculum through the side of a polybag (de Franqueville, 1984) some degree of root damage seems inevitable. Pouring inoculum over the soil around the base of plants 2 weeks after replanting (Flood *et al.*, 1989) ensured minimal, if any, root damage but still gave 100% infection.

In general, nursery trials in Africa (Prendergast, 1963; de Franqueville, 1984; de Franqueville & Renard, 1990) have relied upon destructive sampling of large numbers of inoculated plants after a set period and comparing progenies by the percentage of diseased plants. In contrast, experiments in Europe (Locke & Colhoun, 1974; Ho *et al.*, 1985a, b; Flood *et al.*, 1989) have had to use fewer plants and the growth of inoculated and uninoculated plants for each progeny has been compared.

Whatever the merits of the nursery wilt test (in terms of savings in space and time), the ultimate assessment for wilt resistance is still the field trial, where disease resistance (and other characteristics) can be measured under plantation conditions over a period of years.

In Zaire and the Ivory Coast the development of disease in field palms has been monitored by use of a "wilt census", in which palms are rated as either healthy or wilted on the basis of leaf symptoms (de Franqueville & de Greef, 1987; de Franqueville & Renard, 1990). As with nursery trials, crosses are compared by use of a "wilt index", which is based on the percentage infection of each cross divided by the percentage infection for the whole trial. This index is thought to reduce the effects of environmental and age differences between trials (de Franqueville & de Greef, 1987).

There have been several attempts to establish the validity of the nursery test by comparing results from

nursery and field trials. Renard *et al* (1972) and de Franqueville (1984) observed a good correlation between nursery and field results. However some progenies showed resistance in the nursery trial that was not observed in the field (de Franqueville, 1984). Therefore de Franqueville (1984) suggested that nursery trials should only be used to discard susceptible material and that erratic results in some trials were due to ineffective inoculation techniques.

A large programme of nursery wilt testing was initiated in the Ivory Coast, from 1970 to 1972, and de Franqeville and Renard (1990) claimed that this was responsible for a drop (from 25% to 15%) in the incidence of wilt in field planting. However, from their data, most of this improvement appears to have occurred from 1967 to 1970, which was before any nursery screened material was planted.

Nursery and field trials have been invaluable for the selection of more resistant progenies. However, the logical development and utilization of host resistance would be assisted by an understanding of the genetics of the host/pathogen interaction.

For example, the reliability of the nursery test depends on the use of a pathogen isolate or isolates that are representative of the area where palms are to be planted. In particular, it is important that although isolates may vary in the degree of symptoms they induce

(aggressiveness) they do not significantly differ in their ranking for resistance of different palm crosses (virulence). Therefore, there should be no differential interaction between isolates and palm crosses (Renard *et al.*, 1972; de Franqueville, 1991).

For this reason several studies of pathogen variability have been performed. Prendergast (1957) found no differences in isolates obtained from diseased palms in and around Cowan estate in Nigeria, and although Obuekwe and Osagie (1989) reported differences in symptom development induced by isolates from Nigeria, Zaire and Cameroon there appeared to be no isolate/cross interaction. Furthermore, no difference in virulence was found for isolates obtained from palms exhibiting the acute or chronic forms of wilt in the Ivory Coast (de Franqueville, 1991). The failure to observe differences in pathogen virulence in nursery trials led de Franqueville (1991) to state that the selection of resistant material in the Ivory Coast based on inoculation tests with one or a few isolates was valid.

However , there is evidence that significant pathogen variability does exist between different areas. Disease resistant material imported into Nigeria from the Cameroon and Ivory Coast were reported to be much more susceptible than resistant material selected using Nigerian isolates (Aderungboye, 1981; Oritsejafor, 1989). In particular, the material selected as resistant in the Ivory Coast had the highest incidence of wilt (47%) anywhere in Nigeria

(Oritsejafor, 1989). Conversely, material selected as resistant against Nigerian isolates has proved susceptible in the Ivory Coast (Renard, 1991, pers comm), and material resistant to Zairean isolates was susceptible to an isolate from Brazil (Flood *et al.*, 1992).

Field and nursery trials have also been used to develop theories on the genetics and nature of host resistance, but, as above, contradictory theories have evolved.

Prendergast (1963) and Meunier *et al.*, (1979) reported that some palms in every cross tested became diseased in nursery trials, and proposed (without direct evidence) that this lack of immunity indicated that resistance was polygenic. However, it could also reflect the rigorous nature of the nursery trial, and in other wilt diseases, where resistance is controlled by strong single genes, a similar breakdown of immunity has been reported when plants are inoculated in too rigorous a manner (Beckman, 1987).

More direct evidence for polygenic control of resistance was provided by Meunier *et al* (1979) who crossed 8 palm lines, ranging from highly susceptible to resistant, and tested the resistance of the progeny in a nursery trial. They concluded that there were both resistance and susceptibility genes that were present in high numbers and were additive in effect.

This theory of polygenic resistance led workers at I.R.H.O. (Institut de Recherches pour les Huiles et

Oleagineux) to conclude that within a cross, individual palms would have a similar number of resistance genes. They assumed that the failure of some inoculated palms to exhibit symptoms reflected the "threshold" nature of disease resistance, rather than segregation of resistance genes within a cross. Therefore the relative resistance of a cross could be estimated by the percentage of inoculated plants that became infected (Meunier *et al.*, 1979).

An alternative theory for the inheritance of resistance was suggested by de Franqueville & de Greef (1987). They studied the development of disease in field palms in the genealogical block at Binga in Zaire, where they observed a wide range of losses in different progenies (from 0 to 90%). They discovered that in crosses between the most resistant and susceptible palms, the level of resistance did not appear to have been inherited in an additive manner, and that some form of segregation had occurred. They concluded that resistance was controlled by 2 genes, which conferred different types of resistance, segregated independently and had an additive effect. For each gene, one allele governed resistance and the other susceptibility. Thus, a heterozygote had an intermediate level of resistance conferred through semi-dominance. Significantly, this theory suggests that resistance genes would be unevenly distributed between palms within a cross, and large differences in the nursery wilt resistance of clonal lines produced from trees of the same cross have been reported (de Franqueville, Diabate, Renard

& Jaquemard, 1991). These authors however, did not believe that any conclusions on the genetics of disease resistance could be drawn from these results (de Franqueville, 1991, pers comm).

Further support for simple genetic control of resistance was found with some lines of *Elaeis oleifera* (the South American oil palm) which showed immunity to infection at the nursery stage (Renard, Noiret & Meunier, 1980). Since this immunity had never been observed in any *E. guineensis* crosses, yet could be transmitted to some *guineensis* x *oleifera* hybrids they concluded that the resistance characteristic was different to those of *E. guineensis* and was due to "simple genetic determinism".

In conclusion, the genetic mechanisms underlying disease resistance in oil palm remain unclear. The contradiction between the theories concerning polygenic and 2 gene inheritance may be partly explained by differences in methodology (respectively nursery *vs* field trials) and by the possible wider genetic base at Binga on which de Greef & de Franqueville based their observations.

4.2 RESULTS

Introduction

As outlined in the introduction, the selection of disease resistant palm crosses or clones is carried out in two stages. In the nursery wilt trial, young artificially inoculated palms are rated as resistant or susceptible on the basis of symptom development. Then material regarded as resistant in these nursery trials are incorporated into field trials where disease development (and other characteristics) can be assessed over a period of years.

This section describes experiments that were designed to improve current methods in both nursery and field trials. In the nursery experiments, procedures likely to increase the level of infection were investigated as were a range of alternative disease assessment methods. The accuracy of the wilt census of field palms was compared with a new technique for sampling mature palms.

4.3. Improvements to Nursery Wilt Test Procedures

Results of nursery wilt trials at the research station at Binga in Zaire (jointly run by Plantation Lever au Zaire and Societe Culture au Zaire) were regarded as unsatisfactory, with frequently < 30% of plants of susceptible crosses becoming infected (Van Amstel, 1987, pers comm). Following a visit by the author and Dr J Flood to Binga (November, 1987), a major cause of these poor results was thought to be the use of non-standardised inoculum. Fungal isolates obtained from diseased plants were assumed to be Foe, but since isolates were cultured

on a non-selective medium and not examined microscopically some experiments may have been inoculated with a non-pathogenic isolate of *F. oxysporum* or even another species (Flood & Mepsted, 1988). We recommended the use of a standardised inoculum (F3), which was considered to be representative of Zairian isolates, and was originally isolated at Binga. It was further recommended that the isolate (single spore culture) be stored in sterile soil at 7°C to avoid mutation (2.2). This procedure is now adopted at Binga.

However, further improvements to the efficiency and repeatability of the wilt tests were considered possible. Therefore experiments were initiated here to compare the standard nursery inoculation method (de Franqueville, 1984) with (1) higher inoculum levels (Locke & Coulhoun, 1974), (2) applied at an earlier stage (Renard *et al.*, 1972) and (3) a wider range of destructive and non-destructive assessment methods.

4.3.1. Soil Inoculation of Seedling Palms.

A total of 6 experiments were performed, each representing one seedling cross of known parental susceptibility (Table 31); crosses are referred to by their experimental numbers (1 to 6).

Seeds were propagated and inoculated as previously described (2.1, 2.2). In all experiments, plants were inoculated at both high and low spore concentration at the

2 leaf stage, and (where possible) 1, 3 and 4 leaf stage inoculations at high spore concentration (Table 32).

Table 31: Details of Seedling Crosses.

Seedling Cross	Parental Cross	Parental Wilt Indices *
1	F43/21 x A24/24	70 x 39
2	F43/21 x A25/14	70 x 155
3	E38/7 x F16/25	54 x 42
4	E98/34 x E81/29	103 x 41
5	E107/22 x A112/26	49 x 46
6	E118/18 x A112/26	49 x 46

* The field wilt index used here is the % wilted palms of a cross divided by the % wilted palms of all crosses in that trial, x 100.

Table 32: Details of Host Age when Inoculated and Inoculum Concentration.

	Host Age Inoculum Concentration *							
Seedling Cross	1st Leaf Low	1st Leaf High	2nd Leaf Low	2nd Leaf High	3rd Leaf Low	3rd Leaf High	4th Leaf Low	4th Leaf High
1	-	+	+	+	-	+	-	-
2	-	+	+	+	-	+	-	-
3	-	+	+	+	-	+	-	-
4	-	-	+	+	-	+	-	+
5	-	-	+	+	-	-	-	-
6	-	-	+	+	-	-	-	-

* Low concentration = 10 ml of 3.3×10^6 spores ml^{-1}
High concentration = 10 ml of 3.3×10^7 spores ml^{-1}
+ inoculation performed, - inoculation not performed.
Seedling crosses 1-3, 16 replicate plants per treatment; crosses 4-6, 14 replicates per treatment.

Due to variable, and often low, seed germination it was not possible to stagger germination over time, and consequently seedlings inoculated at different stages of development were inoculated at different times. However, all results were expressed relative to days post-inoculation at the 2 leaf stage (P2LI), which is the standard procedure in this laboratory.

Every 28 days (P2LI), the height and transpiration rate of each plant was assessed (2.3.1.a & 2.3.4.a) and at the end of experiments measurements of dry weight of aerial tissue and browning of bulb stem tissue were taken (2.3.1.e & 2.3.2.b).

Experiments 1 to 3, which were initiated before experiments 4 to 6, were destructively harvested 205 days P2LI but experiments 4 to 6 were not finally harvested till 289 days P2LI because of a slower development of symptoms, This delay was justified because comparisons between inoculation and assessment methods could only be performed when adequate symptom development had occurred. As a result, direct comparisons between the 2 groups were only possible for height data, which was collected at standard intervals. Transpiration data, although collected at the same intervals, were not comparable because vapour pressure deficits were not controlled.

4.3.1.a. Comparison of Disease Assessment Methods.

The relative sensitivity of each method was evaluated by comparing the frequency with which that method demonstrated significant differences between palms

inoculated at the 2 leaf stage and uninoculated controls (Table 33a,b).

Table 33a: Comparison of Methods of Disease Assessment by Measurements of Growth and Transpiration.

Experiment Number and Inoculum Concentration	Infected Plants as a Percentage of Uninoculated Control		
	Height	Dry Weight $	Transpiration
1 High	86.3	84.9	77.9
2 High	78.5*	72.5*	62.0*
3 High	86.8	81.4	80.6
4 High	89.4	83.1	80.7
5 High	92.2	84.0	85.2
6 High	94.0	103.5	107.0
1 Low	86.3	88.1	86.2
2 Low	84.7	82.2	85.5
3 Low	97.0	100.3	97.3
4 Low	93.8	93.9	107.4
5 Low	98.7	95.9	89.1
6 Low	96.8	98.7	95.8

Plants were inoculated at the 2 leaf stage. For experiments 1-3 and 4-6 values are the mean of 16 or 14 plants respectively.

For experiments 1-3, height and transpiration measurements were taken 194 days post inoculation, dry weight 205 days post inoculation.
For experiments 4-6, height and transpiration measurements were taken 285 days post inoculation, dry weight 289 days post inoculation.

* Significantly different to uninoculated control ($P < 0.05$; Anova, Bonferroni's test).

$ Dry weight of aerial tissue.

Table 33b: Comparison of Methods of Disease Assessment by Measurements of Internal Symptoms.

Experiment Number and Inoculum Concentration	Percentage Bulb Stem Browning	Ratio of Plants With : Without Browning of Bulb Tissue
1 High	27.1 *	14 : 2 *
2 High	60.2 *	11 : 5 *
3 High	22.4 *	11 : 5 *
4 High	16.1 *	9 : 5 *
5 High	14.0 *	9 : 5 *
6 High	8.0	5 : 9 *
1 Low	12.9	11 : 5 *
2 Low	30.8 *	8 : 8 *
3 Low	6.2	7 : 9 *
4 Low	12.3	8 : 6 *
5 Low	6.3	5 : 9 *
6 Low	1.5	4 : 8 *

Plants were inoculated at the 2 leaf stage, and assessed 205 (experiments 1-3) or 285 (experiments 4-6) days post inoculation.

* Significantly different to uninoculated control ($P < 0.05$; percentage browning of stem tissue:- Mann Whitney U test; frequency of stem browning :- Chi-squared, Fisher's Exact test).
Note, the U test is not strictly appropriate for the percentage stem browning data, because control data were all zero.

Although infection generally resulted in a reduction of height, dry weight, and transpiration, these reductions were only statistically significant with cross 2 inoculated at high dose (Table 33a).

In contrast, measurements of the percentage browning of stem tissue demonstrated significant differences on 50% of occasions, whilst analysis of the frequency of browning of stem tissue always produced significant results (Table 33b).

4.3.1.b. Effect of Host Age at Inoculation on Symptom Development.

As previously described, plants inoculated at different ages, were inoculated at different times. Thus, in experiment one, final assessment of 1, 2 and 3 leaf inoculated plants was respectively performed 225, 204 and 166 days post inoculation, which made direct comparisons impossible. However, in a nursery trial it is the total time (both pre and post inoculation) necessary for symptom development that is of greater importance, and therefore it is valid to harvest all treatments at the same time.

As with comparisons of assessment methods, the effect of timing of inoculation was calculated by comparing the frequency with which symptom development was significantly different to uninoculated controls (Table 34).

Earlier inoculation resulted in more significant symptom development; for example, in experiments 1-3, plants inoculated at the 1, 2 and 3 leaf stage were assessed as significantly different to controls on 80%, 60% and 20% of occasions respectively (Table 34).

The extent to which this result reflected a reduction in susceptibility with increasing plant age, rather than a

reduction in time from inoculation to assessment, was estimated by calculating the time from inoculation to a 15% reduction in mean height (Table 35). The age at inoculation had no effect on cross 2 (the most susceptible cross) whilst crosses 1 and 3 were more stunted when inoculated as younger plants.

Table 34: Summary of the Effect on Symptom Development of Host Age at the Time of Inoculation.

	Number of Assessments Methods Significantly Different to Uninoculated Control ($P < 0.05$) $			
Experiment	Leaf Stage when Inoculated			
	1	2	3	4
1	5	2	1	-
2	5	5	1	-
3	2	2	1	-
4	-	1	1	1
5	-	3	-	-
6	-	1	-	-

- inoculation not performed, all other plants were inoculated at high dose.

$ Five assessment methods were used:- plant height, transpiration, dry weight of aerial tissue, % of stem browning and frequency of stem browning.

In experiments 1-3 :- Height and transpiration measurements were 194 days P2LI, other assessments were 204 days P2LI. One and 3 leaf stage inoculation were respectively 21 days before and 38 days after P2LI.

In experiments 4-6 :- Height and transpiration measurements were 285 days P2LI, other assessments were 289 days P2LI. Three and 4 leaf stage inoculation were respectively 21 and 38 days after P2LI.

Table 35: Effect of Age at Inoculation on the Rate of Symptom Development.

	Time (Days) from Inoculation to a 15% Reduction in Plant Height *		
	Growth Stage when Inoculated		
Experiment	1 Leaf	2 Leaf	3 Leaf
1	120	140	>156 $
2	131	122	125
3	83	180	>156 $

All plants were inoculated at high dose.

* Values were calculated from graphs of mean height over time and compared with the height of uninoculated controls.

$ Plants <15% stunted at the end of the experiment (156 days after 3 leaf stage inoculation).

4.3.1.c. Effect of Inoculum Concentration on Symptom Development.

High dose inoculation at the 2 leaf stage nearly always produced greater symptom development than low dose inoculation (Table 33a,b), but within each cross none of these differences were statistically significant.

However it was possible to compare low and high dose inoculation by 2 way analysis of variance of height data at comparable times post inoculation (crosses 1-3, 194 days; crosses 4-6, 201 days). To compensate for differences in growth between crosses, the height of inoculated plants was converted to a percentage of the mean height of the controls for that cross. This analysis demonstrated that high dose inoculation caused an average

reduction in height of 12.8% compared with 7% at low dose, and this difference was highly significant ($P < 0.01$).

The adoption of such a high dose of inoculum at Binga (33 times the current rate), in combination with the large number of plants regularly inoculated, would present severe logistical problems with the present shake culture inoculum production method. Therefore a new technique, suitable for the facilities at Binga and capable of producing adequate inoculum, was developed (Appendix 3).

4.3.1.d. Comparisons Between Different Crosses.

The main purpose of a nursery trial (although not of these experiments) is to make comparisons of symptom development in different crosses. However, for the reasons previously discussed, comparisons of final height and transpiration data between the two groups of experiments (1-3 and 4-6) were not possible. However, comparisons were possible for height data for palms inoculated at the 2 leaf stage (Table 36).

For these data, the only significant difference was between the most susceptible (cross 2) and the most resistant material (cross 6). The susceptibility of cross 2 could be predicted from the high wilt index of one of its parents but no obvious trend discerned between stunting and parental wilt indices for the other crosses (Tables 31 & 36)

Table 36: Comparison of Stunting of Crosses Inoculated at the Two Leaf Stage.

Cross	Height *
2	81.6 a
1	86.3 ab
3	91.9 ab
5	91.9 ab
4	92.9 ab
6	96.7 b

* For each cross, height is the percentage of the mean height for uninoculated palms of that cross, and is the mean of both low and high dose inoculation methods. Numbers with the same letter and not significantly different ($P > 0.05$, 2 way Anova).

In summary, application of increased inoculum to younger plants induced earlier and more severe symptoms, and assessments based on browning of stem tissue produced the most significant results, but required destructive harvesting. A higher level of replication would probably increase the ability of the test to distinguish between resistant and susceptible crosses. Alternatively, this failure to differentiate may reflect a lack of significant differences between crosses.

The opportunity to test some of these conclusions was provided by the provision of sufficient numbers of clonal palms.

4.3.2. Soil Inoculation of Clonal Plants.

Wilt testing of clonal palms at the nursery stage has been performed at Binga and Yaligimba in Zaire since 1984. Unfortunately, the delicate nature of this material combined with transport problems in Zaire has frequently resulted in death of plantlets during hardening off or after transplanting into the nursery (Van Amstel. 1988, pers comm). Although, following our recommendations, the reliability of the nursery wilt test has improved (Nyanduza, 1989, pers comm) a study of the feasibility of conducting glasshouse tests on clonal material was initiated, with a view to conducting such tests in the U.K..

Therefore , based on the results of screening trials with seedlings (see previous section) a wilt test on clonal palms was performed using more replication, high dose inoculation, an improved assessment of leaf stunting and incorporating some clones of known resistance and susceptibility.

Fifty plants of 7 clones (Table 37) were hardened off and propagated as previously described (2.1.). Three weeks after transplanting, these plants were soil inoculated at high dose (2.2.); 24 inoculated and uninoculated plants were used for each clone.

In the experiments on seedlings leaf stunting was assessed by measuring plant height. However, such measurements underestimated stunting when the youngest fully expanded leaf was shorter than the previous leaf.

Therefore, in this experiment stunting was assessed by measuring the height of the youngest fully expanded leaf. To compensate for differences in growth between clones, the leaf height of each inoculated plant was converted to a percentage of mean height of controls of that clone.

Table 37: Details of Clonal Material for Wilt Testing.

Clone	Ortet Resistance Grade *	Previous Wilt Test Result $
136.5	R	S
137.10	S	S
5609	?	S
28.5	R	R
188.2	?	?
174.3	R	?

* The ortet is the palm from which clonal material was obtained. Resistance grade is based on the progeny from which the ortet came.
$ Based on the results of nursery or field trials in Zaire.
S = susceptible, R = resistant, ? = unknown.

Height measurements were made at 28 day intervals from 4 months post inoculation (Tables 38 & 39). These observations were continued until 7 months post inoculation when plants were assessed by measurements of leaf chlorosis, browning of stem tissue and quantitative reisolation of the pathogen (2.3.2.a., 2.3.2.b., 2.3.3.) (Tables 40 & 41).

4.3.2.a. Comparison between Clones.

Measurements of leaf stunting (Table 38) demonstrated good symptom development in this trial, with most clones 30 to 40% stunted by the end of the experiment. These measurements clearly demonstrated clone 28 to be the most resistant (confirming results of previous trials, Table 37) and 174 the most susceptible.

Table 38: A Comparison of Leaf Stunting in Inoculated Clones.

	Mean Height of Youngest Fully Expanded Leaf *			
	Days Post Inoculation			
Clone	112	140	168	196
28.5	93.0 a	88.1 a	85.6 a	81.2 a
137.1	96.3 a	87.7 a	76.3 ab	63.2 ab
5609	84.3 ab	76.4 ab	68.8 b	62.4 ab
4	79.2 ab	68.3 ab	71.7 b	66.9 ab
136.5	83.3 ab	66.4 b	65.0 bc	64.6 ab
188.2	70.7 b	62.4 b	64.1 bc	62.1 ab
174.3	71.5 b	61.9 b	53.7 c	48.0 b

* For each clone height is expressed as a percentage of the mean for uninoculated palms of that clone.

Within each column numbers with the same letter are not significantly different ($P > 0.05$, Anova, Bonferroni's test).

The most significant differences between clones occurred at 168 days post inoculation; by 196 days the increased variance of the data, combined with the statistical test used, decreased the significance of these results. The response of some clones varied during the

course of the experiment, for example, clone 137 appeared comparatively resistant at 112 and 140 days but could not be distinguished from more susceptible clones by the end of the experiment.

The prime function of a wilt test is to establish the resistance of previously untested material, and this was done by comparing each clone against a clone of known resistance (clone 28, Table 37) or susceptibility (clone 174); the susceptibility of clone 174 was solely based on the results of this trial. This analysis (using a nonparametric test which was better suited to the data) demonstrated that at the end of the trial all clones were significantly more susceptible (stunted) than 28 and that clones 28, 137, 4, and 136 were significantly more resistant (less stunted) than 174 (Table 39).

Table 39: A Comparison of Leaf Stunting of Inoculated Clones with a Standard Resistant (28) and Standard Susceptible (174).

	Days Post Inoculation			
	112	140	168	196
Clones significantly more stunted than 28	188 174 4	188 174 4 136	188 174 4 136 5609	188 174 4 136 5609 137
Clones significantly less stunted than 174	28 137 5609	28 137 5609	28 137 5609 4	28 137 4 136

Significant differences based on Mann Whitney U test ($P < 0.05$).

Clone 28 was always the most resistant using the other assessment criteria of leaf chlorosis, pathogen colonisation and stem browning (Tables 40 & 41). But the ranking of other clones varied depending on the assessment method employed. For example, clone 4 had significantly less leaf chlorosis than clone 174 and yet a significantly higher level of colonisation per bulb (Table 40). These variations suggested that either there were no great differences between these clones and/or that different assessment methods measure different resistance or susceptibility characteristics.

Table 40: Colonisation of Clonal Plants.

	Pathogen Reisolation from Bulb Stem Tissue		
Clone	Log C.F.U.s per g Fresh Weight	Log C.F.U.s per Bulb *	Ratio of Plants With:Without Reisolation
28	1.66 a	1.54 a	14 : 10 a
137	3.11 b	3.10 bc	24 : 0 b
5609	2.90 b	2.38 ab	21 : 3 b
4	3.63 b	3.21 c	23 : 1 b
136	3.48 b	3.16 c	23 : 1 b
188	2.99 b	2.78 bc	22 : 2 b
174	2.92 b	2.68 b	24 : 0 b

Figures are the mean of 24 replicate plants, 196 days post inoculation.

Within each column, figures with the same letter are not significantly different ($P > 0.05$, C.F.U.s by STP, reisolation by Chi-squared and Fisher's Exact test)

* calculated from the previous column divided by the weight of bulb stem tissue.

Table 41: Leaf Chlorosis and Browning of Stem Tissue in Clonal Plants.

Clone	Leaf Chlorosis	Percentage Browning of Bulb Stem Tissue	Ratio of Bulbs With:Without Browning of Stem Tissue
28	1.08 a	18.0 a	13 : 11 a
137	1.38 ab	46.2 b	21 : 2 b
5609	2.88 d	57.0 b	21 : 3 b
4	1.63 b	40.2 b	22 : 2 b
136	3.04 d	77.8 c	23 : 1 b
188	2.21 c	45.2 b	24 : 2 b
174	2.42 cd	53.5 b	24 : 0 b

Figures are the mean of 24 replicate plants, 196 days post inoculation.

Within each column figures with the same letter are not significantly different ($P > 0.05$, chlorosis and percentage browning by STP, frequency of browning by Chi-squared and Fisher's Exact test).

4.3.2.b. Comparisons between Assessment Techniques.

At the final harvest, the largest number of significant differences were obtained by measurement of leaf chlorosis, which suggested that this criterion is a better non-destructive method of assessment than leaf height measurements. However, 168 days after inoculation, height measurements produced a comparable number of significant differences. This observation may be partly explained by the increased variance of height data by 196 days combined with the Anova analysis of this data.

Variation in symptom development with time, combined with occasional significantly different rankings of clones

obtained by different assessments (Tables 40 & 41, see leaf chlorosis & cfus per bulb for clones 4 & 174) suggested that the measured symptoms may be unrelated. Thus, a severely stunted palm may not have a high degree of browning of stem tissue. However, if no correlation existed between these symptoms, which, if any, assessment method most accurately measures disease resistance or susceptibility?

To investigate the degree of association between different symptoms, correlation analysis was performed on the final assessment for the clonal palms (Table 42). The purpose of this analysis was to test for association between disease symptoms and thus only data from infected palms (where the pathogen was isolated from stem tissue) were used.

Importantly, nearly all data sets were normally distributed, which indicated that correlations were linear, i.e. one set of symptoms did not "tail off" above a certain level. However, in order to accommodate the few non normal data sets, Spearman's test for rank correlations was performed (Seigel, 1956; Sokal & Rohlf, 1981).

Table 42: Correlations between Final Assessment Methods for Infected Clonal Palms.

Assessment Correlation	Correlation Coefficient for each Clone $ All	28	137	4
Height/chlorosis	-0.261**	-0.497ns	-0.480ns	-0.353ns
Height/% browning	-0.316**	-0.716**	-0.474*	-0.431*
Height/cfus/g	0.068ns	-0.380ns	0.078ns	0.170ns
Height/cfus/bulb	0.219*	-0.196ns	0.085ns	0.316ns
Chlorosis/% browning	0.524***	0.701**	-0.522ns	0.796**
Chlorosis/cfus/g	0.205*	0.507*	-0.703*	0.438ns
Chlorosis/cfus/bulb	0.082ns	0.502ns	-0.710*	0.370ns
% browning/cfus/g	0.244*	0.526ns	0.446*	0.175ns
% browning/cfus/bulb	0.173ns	0.460ns	0.465*	0.104ns

Assessment Correlation	Correlation Coefficient for each Clone $ 5609	136	188	174
Height/chlorosis	-0.190ns	0.505ns	0.318ns	-
Height/% browning	-0.119ns	0.033ns	-0.295ns	-
Height/cfus/g	0.223ns	0.000ns	0.221ns	-
Height/cfus/bulb	0.441*	0.159ns	0.464*	-
Chlorosis/% browning	0.845**	0.495ns	-0.143ns	-
Chlorosis/cfus/g	0.728*	-0.414ns	0.828**	-
Chlorosis/cfus/bulb	0.533ns	-0.428ns	0.699*	_
% browning/cfus/g	0.178ns	-0.236ns	0.224ns	0.035ns
% browning/cfus/bulb	0.009ns	-0.228ns	0.266ns	-0.022ns

- Data not available.
$ Correlation analysis by Spearman's test for ranked data. ($P < 0.001$ ***, $P < 0.01$ **, $P < 0.05$ *, ns no significant correlation)

Frequently, there was good correlation between the percentage browning of stem tissue and both height (negative correlation) and chlorosis (positive correlation). Chlorosis was also often positively correlated with cfus per g, but with clone 137 the significant correlation was negative. Surprisingly, there was little or no association between cfus per g and height or browning of stem tissue. Similarly, little association was found between cfus per bulb and browning of stem tissue, and yet there were some significant correlations between cfus per bulb and height, which probably indicate an association between bulb size and height.

More significant results were obtained by pooling the data (Table 42, first column) but as positive and negative correlations were sometimes found for the association of the same characteristics, pooling data may not be valid.

In summary, the clonal trial demonstrated that with a high inoculum level and sufficient replication it was possible to distinguish between known resistant and unknown susceptible clones in the UK. Correlation analysis of different symptoms, highlighted disparities between the different assessment methods employed. Nonetheless, by measurement of any of the symptoms it was possible to distinguish between resistant and susceptible material.

This trial of clonal material and the previous seedling experiments took 6 to 7 months to perform and therefore, any procedure that would speed up symptom development

would obviously be advantageous. To investigate this possibility some seedlings of crosses 1 to 6 were subjected to more rigorous inoculation procedures.

4.3.3. The Effect of Severe Inoculation Methods on Symptom Development

These experiments involved root dip inoculation, root damage prior to inoculation and injection of inoculum directly into the plant, and were designed to accelerate symptom development and to assess the reaction of crosses previously shown to be resistant or susceptible following soil inoculation.

The treatments (applied to 12 replicate palms of each cross [1-6] at the 2 leaf stage) were as follows:-

a) Root dip inoculation: seedling roots were immersed, for 45 minutes, in a suspension of 1×10^6 spores per ml.

b) Root dip inoculation to cut roots: As above but roots were severed (under water and 50 mm from the bulb) and left in the spore suspension for 4 h, to ensure uptake of spores into the xylem.

c) Injection of inoculum into the bulb: a suspension of 8×10^7 spores per ml mixed with an equal volume of agar (0.4 g / 100 ml) was injected in 2 places into the base of each plant bulb using a 1.00 mm O.D. needle and syringe. The agar increased the viscosity of the inoculum, so that any excess could be removed from the bulb surface and did not drain into the soil. This method caused excessive damage to plants of crosses 2 and 3 and was therefore

modified, by using a 0.5 mm O.D. needle and injecting just above the roots. This measure reduced damage to the growing point, which at this stage of development is situated in the middle of the bulb.

For each inoculation method, control plants received the same treatment with 1/10 strength sucrose salts solution. The effect of inoculation on plant height, dry weight and the frequency of stem browning were assessed as previously described (2.3.1.a., 2.3.1.e., 2.3.2.b.).

4.3.3.a. Comparisons between Inoculation Methods.

With one exception (dry weight of cut-root inoculated plants of cross 3) all root dip and cut root inoculations induced symptoms that were significantly different to uninoculated controls (Tables 43-45). The first injection method, used in crosses 2 and 3, also caused appreciable symptom development (Tables 43-45) but resulted in substantial reductions (up to 50%) in the height and dry weight of some control plants. The less damaging injection technique used for crosses 4 to 6 was usually associated with relatively minor symptom development, although there was a significant reduction in height with cross 6 and all 3 crosses showed significant browning of bulb stem tissue (Tables 43-45).

Table 43: Effect of Severe Inoculation Methods on Seedling Height.

	Height as a Percentage of Control		
Seedling Cross	Inoculation Method Root Dip	Cut Root	Injection
1	57.6 **	68.2 *	-
2	47.8 **	-	60.0 *
3	-	81.3 *	68.8
4	46.0 **	69.2 *	97.9
5	49.8 **	58.8 **	93.9
6	59.3 **	48.1 **	83.0 *

Experiments 1-3 and 4-6 were assessed respectively 141 and 112 days post inoculation.
- Inoculation not performed.
* Significantly difference between inoculated plants and uninoculated control (T-test, * $P < 0.05$, ** P , < 0.01, *** $P < 0.005$)

Table 44: Effect of Severe Inoculation Methods on Seedling Dry Weight.

	Dry Weight as a Percentage of Control		
Seedling Cross	Inoculation Method Root Dip	Cut Root	Injection
1	38.6 ***	58.8 **	-
2	36.0 ***	-	49.8
3	-	81.2	70.7
4	34.4 ***	59.7 **	91.7
5	54.8 ***	54.7 **	86.4
6	-	39.9 ***	73.1

Experiments 1-3 and 4-6 were assessed respectively 141 and 112 days post inoculation.
- Inoculation not performed or missing data.
* Significantly difference between inoculated plants and uninoculated control (T-test, * $P < 0.05$, ** P , < 0.01, *** $P < 0.005$).

Table 45: Effect of Severe Inoculation Methods on the Frequency of Browning of Bulb Stem Tissue.

Seedling Cross	Ratio of Plants With : Without Browning of Stem Tissue Inoculation Method Root Dip	Cut Root	Injection
1	12 : 0 *	12 : 0 *	-
2	11 : 1 *	-	12 : 0 *
3	-	10 : 2 *	9 : 3 *
4	12 : 0 *	12 : 0 *	4 : 8 *
5	11 : 1 *	11 : 1 *	3 : 9 *
6	10 : 2 *	12 : 0 *	2 : 10 *

Experiments 1-3 and 4-6 were assessed respectively 141 and 112 days post inoculation.
- Inoculation not performed.
* Significant difference between inoculated plants and relevant control ($P < 0.05$, Chi Squared and Fisher's Exact test).

4.3.3.b. Comparisons Between Crosses.

Direct comparisons between crosses 1 to 3 and 4 to 6 were not possible because they were not assessed at the same time. But within each group, none of the inoculation methods showed any significant differences between crosses. However, in the first group, cross 3 was always the most tolerant and within the second group, cross 6 was frequently (4 out of 8 occasions) the most susceptible (Tables 43-45).

4.3.3.c. Comparison Between Soil Inoculation and Severe Inoculation Methods.

Cut root and root dip inoculations resulted in more rapid and extreme symptom development than with soil

inoculation. For example, 24 out of 180 inoculated plants were dead before the experiments finished, while mortality was rare in soil inoculation experiments. Because plant death reduced the validity of disease assessments, and due to the enhanced rate of symptom development, these experiments were harvested much earlier (141 or 112 days post inoculation) than the soil inoculation experiments (204 or 289 days post inoculation).

As final assessments were performed at different times, direct comparisons between soil inoculation and these experiments were impossible, but comparisons of height data were valid. Thus, 110 days post inoculation, root dip and cut root inoculation induced a 30 to 60% reduction in height, whilst only 2 to 16% stunting was observed in plants that had received high dose soil inoculation at the 2 leaf stage.

Injection inoculation of crosses 1 to 3 was too severe a test, because some control plants were also considerably stunted. However, more careful injection, using method 2, of crosses 4 to 6 caused intermediate levels of stunting (8 to 27%).

The key comparison between soil inoculation and more severe inoculation techniques is the order in which they rank crosses for resistance. For crosses 1 - 3 , both types of inoculation ranked cross 3 as the most resistant. Using soil inoculation, cross 6 was frequently (on 8 out of 10 assessments, Table 33) the most resistant cross; and

where comparisons between all crosses were possible (Table 36) it was the most resistant of all crosses. However, with the more severe inoculation methods cross 6 was frequently the most susceptible (4.3.3.b.)

4.3.4. The Effect of Soil Temperature on the Growth of *F.oxysporum* and on the Infection of Oil Palm Seedlings.

Prendergast (1963) found it necessary to provide shading for seedlings that were root dip inoculated and then immediately planted in the nursery. With the replacement of root dip inoculation by soil inoculation, shading was no longer considered necessary in Zaire (de Franqueville, 1984). However, during a visit, by the author, to Binga (November 1987), very high soil temperatures (> 40 ^{o}C) were recorded in soil adjacent to the bulbs of seedlings in a nursery trial. Further measurements revealed that soil temperatures (1.5 cm below the surface) rose from 23 ^{o}C at 6.30 am to above 40 ^{o}C by 11.00 am and nearly 42 ^{o}C by 2 pm. Maximum temperatures 8 and 15 cm below the surface were respectively 2 and 6 ^{o}C below these values. Soil adjacent to the bulbs of older palms in the nursery (about 1.5 m in height) was 36 ^{o}C at 2 pm, whilst comparable measurements of soil temperature in the plantation were only 22-24 ^{o}C; lower temperatures reflect increasing degrees of shading from larger palms.

Elevated soil temperatures in the nursery, far in excess of plantation soil temperatures, could affect the physiology of both host and pathogen and possibly reduce the level of infection in nursery trials. The effect of

temperature on the growth of Foe and on infection of seedlings was therefore investigated.

4.3.4.a. The Effect of High Temperatures on the Growth of *F. oxysporum in vitro.*

Agar plugs (5 mm diameter) were removed from the margin of a colony of Foe (on PDA) and placed in the centre of petri dishes containing PDA. Four replicate plates were incubated at each of a range of temperatures (15, 20, 25, 30, 32.5, 35 & 40 ^{o}C) for 4 days, when measurements of colony diameter (2 per plate) were taken (Table 46).

The optimum temperature for growth was between 25-30 ^{o}C, with a very rapid reduction in growth at temperatures > 30 ^{o}C and no growth at all at 40 ^{o}C.

Similar experiments were also conducted on with 3 other *formae speciales* (2 temperate, 1 subtropical) of *F. oxysporum (pisi, lycopersici & albedinis)* which produced very similar results to those observed for Foe (Table 47). This suggested that isolates pathogenic to tropical or subtropical crops are not adapted to higher soil temperatures.

Table 46: Effect of Temperature on the Growth of *F. oxysporum f.sp. elaeidis In Vitro* .

Temperature (^{o}C)	Increase in Colony Diameter (mm) $
15	18.1
20	25.6
25	42.0
30	41.2
32.5	21.5
35	2.3
40	0

$ Values represent the increase in size (beyond original diameter of 5 mm) of 4 replicate colonies after 4 days. All values are significantly different ($P < 0.05$, STP).

Table 47: Effect of Temperature on the Growth of four *formae speciales* of *F. oxysporum In Vitro.*

Temperature (^{o}C)	Increase in Colony Diameter $ *forma specialis*			
	elaeidis	*albedinis*	*pisi*	*lycopersici*
15	16.0	14.1	16.3	19.5
20	22.0	21.6	22.5	27.0
25	35.0	31.5	31.9	35.1
30	32.9	29.0	33.1	32.5
35	6.6	10.0	9.8	9.25

Values represent the increase in size (beyond original diameter of 5 mm) of 2 replicate colonies after 4 days.

Although a constant 40 ^{o}C completely inhibited fungal growth, such treatment does not simulate the fluctuating temperatures observed in the nursery. Therefore, the

experiment was repeated with a temperature regime of 16 hrs at 25 ^{o}C followed by 8 hrs at 40 ^{o}C (Table 48). Although the pathogen was not killed by periods of high temperature, its overall growth rate was reduced to approximately 50 % of that of cultures grown at a constant 25 ^{o}C.

Table 48: Effect of Temperature Regime on the growth of *F. oxysporum f. sp. elaeidis in vitro.*

	Colony Diameter (mm) $	
Time (days)	Constant 25 ^{o}C	Alternating 25/40 ^{o}C *
1	8.4 a	8.5 a
2	2.3 a	13.5 b
3	34.5 a	19.2 b
4	44.3 a	25.9 b
5	52.8 a	29.9 b
6	60.8 a	35.8 b

$ Mean of 4 replicate colony diameters.
* From 24 hrs onwards colonies received 8 hrs at 40 ^{o}C then 16 hrs at 25 ^{o}C, temperature cycling was repeated daily.
At each time, figures with the same letter are not significantly different (P > 0.05, T test).

It is apparent that the high soil temperatures observed in the nursery trial could suppress the growth of the pathogen, but high temperatures could also affect host physiology which may influence disease development. Therefore, the effect of high soil temperature on disease development was also examined.

4.3.4.b. The Effect of High Soil Temperature on Symptom Development in Seedlings.

The aim of these experiments was to increase the soil temperature around inoculated and uninoculated palms to a level similar to that recorded at Binga, and observe its effect on symptom development and the growth of palms.

Elevated soil temperatures were achieved by designing a system in which hot water was pumped through polyethylene tubing (15 mm I.D.) coiled around the tops of plant pots. The water was pumped (at 12 l / min) from a 10 l container using a water bath heater and pump (Thermo stirrer 85; Gallenkamp, Loughborough) through parallel lines of tubing each leading to a trough of pots (Fig 10). A soil temperature of *ca* 40 ^{o}C at a depth of 15 mm, adjacent to the palm bulb, was achieved by setting the water bath to 80-85 ^{o}C for 2 hrs, then reducing the temperature to 70-75 ^{o}C. There was < 1 ^{o}C drop in soil temperature between the first (input) and last (output) pots in each trough. The soil was heated from 0900-1700 h 5 days a week and this resulted in soil temperatures at, or near, 40 ^{o}C for the last 5 h of heating.

This regime was started on the day of inoculation and after 12 weeks the maximum soil temperature was reduced to 35 ^{o}C; heating was discontinued 4 weeks later. These temperature reductions were performed to simulate the shading effect observed at Binga as canopy size increased.

Figure 10. Apparatus for Increasing Soil Temperature.

Two soil heating experiments were performed, and on each occasion plants were inoculated with a low dose at the 2 leaf stage, and measurements were made of stem browning and height (2.2, 2.3.2.b, 2.3.1.a).

In the first experiment, using seedlings of cross 4 (Table 31) and assessed 289 days post inoculation, elevated soil temperatures had no effect on the height of inoculated or uninoculated plants, but it did reduce the mean level of stem browning (from 12.3 to 3.8 %) and frequency of stem browning (from 8/14 to 5/14 replicates). However none of these differences were statistically significant.

The lack of significant reductions in disease levels may have been due to the relative resistance of cross 4 (Table 36); therefore the experiment was repeated with UF 4 clonal palms, which were known to be susceptible (Table 41). Soil heating significantly reduced both the degree and frequency of browning of stem tissue (Table 49).

Table 49: Effect of Elevated Soil Temperature on Symptom Development in Susceptible UF 4 Clonal Palms

Treatment	Mean Percentage Browning of Stem Tissue	Ratio of Palms With : Without Browning of Stem Tissue
Uninoculated	0	0 : 14 a
Uninoculated, Soil Heated	0	0 : 14 a
Inoculated	50.8 a	13 : 1 b
Inoculated Soil Heated	10.7 b	2 : 14 a

Palms were assessed 292 days post inoculation.

Within each column, figures with the same letter are not significantly different ($P > 0.05$; % browning Mann Whitney U test, Frequency of browning Chi Squared and Fisher's Exact test).

Although improvements have been made to the nursery wilt test, the ultimate assessment of disease resistance is still the field trial.

4.4. The Assessment of *Fusarium* Wilt in Field Palms.

The production of resistant breeding material depends upon a field census which allows for the selection of crosses or clones with a lower than average incidence of wilt. However, this wilt census is based entirely upon the assessment of visible symptoms in field palms. Unfortunately, as foliar symptoms of *Fusarium* wilt can easily be confused with those caused by other diseases, such as *Ganoderma* and *Armillaria* trunk rot (Wardlaw, 1950), the accuracy of the census may be questionable. In contrast to foliar symptoms, the presence of brown vessels in the vascular strands of the trunk is diagnostic for

Fusarium wilt (Wardlaw, 1950). However, in the past the examination of trunk vessels necessitated the destructive sampling of whole palms, which precluded the continued monitoring of individual trees and was too damaging to be acceptable for more than a few palms. Thus, a non-destructive method for the detection of brown xylem elements within trunks was required. A tree increment borer, designed to remove cores from the inside of the trunk, was taken to Binga for evaluation as an alternative approach to symptom assessment.

4.4.a. Comparison between Disease Assessment by Observation of Internal or External Symptoms.

The increment borer was used to extract trunk cores as previously described (2.3.2.c.). Field palms planted 6, 12 or 16 years previously were selected for investigation. These palms were classified as resistant or susceptible depending on the percentage of that cross or clone with disease symptoms (Table 51, footnote). Four trunk cores were removed from the base (*ca.* 500 mm above soil level) of each 6 yr old palm and 3 cores from the base, middle and top of the trunk of older trees. Cores were examined with a hand lens for the presence and severity of vascular browning (2.3.2.c) and the pathogen was isolated from surface sterilised cores (2.3.3). These results were compared with the wilt census, which had been conducted within the previous month.

The auger effectively removed samples from palm trunks and vascular browning in cores was easily observed (Fig

2c). When reisolation was attempted, *F. oxysporum* was found in > 88% of samples containing brown vessels and therefore vascular discoloration was confirmed as diagnostic for infection by Foe. Cores removed from the trunk base and middle contained more brown vessels than those removed from the top of the palm, although this difference was not statistically significant (Table 50).

Table 50: Xylem Vessel Occlusion in Trunk cores from Diseased Palms.

Position in Ttrunk of Cores *	Percentage of Xylem Vvessels Affected $
Top	12.8
Middle	27.3
Base	32.4

Values represent the mean of eight 12 yr old palms and are not significantly different (P > 0.05, STP test)
* At each height, 3 cores were extracted from each palm.
$ Affected vessels contained gels, tyloses and hyphae, surrounding parenchyma cells were brown.

The comparison between the wilt census and borer survey (Table 51) demonstrated that for palms regarded as wilted in the census there was a close agreement between the 2 techniques. Thus, 26 out of 29 "wilted" palms contained brown vascular tissue in the trunk. Also, among trees not considered wilted in the census, good agreement was found for 12 and 16 yr old "resistant" material and 6 yr old symptomless randomly selected palms, i.e. no brown vessels were found in the trunk of any of the 20 palms in this group. However, of the remaining palms with no external

symptoms, comprising 6 and 12 yr old "susceptible" crosses and 16 yr old palms of unknown resistance (cross 29), 11 out of 21 trees contained brown vascular tissue in the trunk.

Table 51: Comparison of the Wilt Census with Auger Survey.

Age of Palms (Years)	Cross or Clone	Wilt Resistant(R) Susceptible(S) *	Wilt Census Results $	Auger Results +	
				Brown Vessels	No brown Vessels
6	UF4	S	Wilted	8	0
6	Random Selection	?	Wilted	4	1
12	109	S	Wilted	7	0
12	106	R	Wilted	1	0
12	29	?	Wilted	6	2
6	UF4	S	Not Wilted	2	3
6	Random Selection	?	Not Wilted	0	11
12	109	S	Not Wilted	5	3
12	106	R	Not Wilted	0	3
16	29	?	Not Wilted	4	4
16	Dumpy x	R	Not Wilted	0	6

* Clone UF4 had 64% wilted or dead palms
Cross 106 had 12% wilted or dead palms
Cross 109 had 50% wilted or dead palms
Dumpy x had 0% wilted or dead palms.

$ Palms categorised as diseased or healthy based upon foliar symptoms.
+ Number of palms with or without brown vessels in the trunk.

Examination of cores from cross 109 and clone UF4 demonstrated that the percentage of vessel browning

(2.3.2.a) from symptomless but infected palms was significantly less than in obviously wilted tress (means 16.6% and 45.1% respectively, $P < 0.01$, T test). For further analysis of the relationship between vessel blockage and symptom development see 3.3.9.

Since Foe is regarded as a root infecting pathogen, the borer survey was extended to a compare root and trunk vascular occlusion in order to determine if root sampling could also be used as an indicator of infection.

4.4.b. Comparison of Root and Trunk Vascular Occlusion in Field Palms.

Ten samples from primary roots were taken from the base of 6 year old UF4 palms, and after sectioning and microscopic examination the percentage of infected vessels from each palm was calculated. These results were then compared with estimates of trunk xylem occlusion obtained by examination of borer cores from the same palms.

Low and similar levels of xylem occlusion occurred in roots from both healthy and diseased palms (Table 52). This demonstrated that root sampling could not be used for disease assessment and that the vascular occlusion observed in these roots was probably not due to the pathogen. Furthermore, in diseased palms, vascular occlusion was confined almost entirely to the stem.

Table 52: Occlusion of Xylem Vessels in the Roots and Trunks of Healthy and Diseased Palms.

	Percentage of Xylem Vessels Affected *	
	Uninfected Palms $	Infected Palms $
Roots +	3.2 a	3.85 a
Trunk Θ	0 a	42.7 b

* Affected vessels contain gels and/or tyloses.
$ Based on external symptoms and browning of trunk xylem vessels in 13, 6 year old UF4 clonal palms
+ Mean of all vessels in 10 primary roots from the base of each palm.
Θ Mean of 4 cores per trunk, (> 20 vessels were examined in each core)
Within each column, numbers with the same letter are not significantly different ($P < 0.01$, Mann Whitney U test)

In summary, the increment borer was an effective tool for the non-destructive evaluation of trunk colonisation. and could also be used to obtain trunk samples, from which Foe could be isolated. In particular, it was possible to obtain isolates from diseased palms of clone 28 that was known to be disease resistant, and the virulence of these isolates could then be tested.

4.5. Pathogen Variability and the Evaluation of Disease Resistance

Although, as described above, it may be possible to improve the efficiency of nursery trials, the validity of the test is dependent on the inoculation of plants with a standard and representative isolate of the pathogen. However, as mentioned in the introduction, there is evidence for differences in aggressiveness of isolates

within a region and variation in virulence of isolates between regions. Thus resistance may be ineffective if the "resistant" palm line encounters isolates that are more aggressive or virulent than the one against which it was originally tested.

As discussed previously (4.1) no clones and few crosses exhibit zero infection in the field or nursery test, and this has led to the suggestion that resistance is of a "threshold" nature. Alternatively, it is possible that within a resistant cross or clone, those palms that develop wilt are more susceptible, or have encountered a more aggressive or virulent isolate of the pathogen.

In experiments with the resistant clone 28, diseased palms were sometimes observed in both nursery and field trials. Assuming variation in resistance within a clone does not occur and all plants were clone 28, then such palms either reflect the threshold nature of resistance or variation in the pathogen.

The latter possibility can easily be tested because isolates obtained from these palms would be more aggressive or virulent to other clones 28 plants. Therefore, clone 28 plants were inoculated with one of the following isolates :-

A). F3; the standard isolate used in nursery trials, originating from a diseased palm at Binga.

B). "Recycled" F3; an isolated obtained from a highly diseased clone 28 palm inoculated with F3 in a nursery trial at Bath.

C). S1 or S2; isolates from severely diseased clone 28 field palms at Binga.

Plantlets were inoculated at both low and high dose (2.2). To allow for full symptom development final assessments of leaf chlorosis and stem browning (2.3.2.a & b) were not performed until 340 days post inoculation (Table 53).

As in previous experiments with this clone, most palms (96 out of 112) remained uninfected, whilst a few (3) were severely affected (> 50% browning of stem tissue). There were no statistically significant differences in symptom development between any of the isolates (Table 53).

These results indicate that infection of nursery palms of this resistant clone was not due to an increase in pathogen virulence/aggressiveness and that field plantings had not selected out a more virulent or aggressive isolate from the plantation population of Foe

Table 53: The Effect of Inoculum Source on Symptom Development in Resistant (UF28) Clonal Palms.

Pathogen Isolate and Dose $	Mean Leaf Chlorosis	Mean % Browning of Stem Tissue	Ratio of Plants With:without Browning of Stem Tissue
Control	0.5	0	0 : 14
F3 Low	0.71	4.32	2 : 12
F3 High	0.86	4.04	3 : 11
Recycled F3 Low	0.64	0	0 : 14
Recycled F3 High	0.79	2.86	1 : 13
S1 Low	0.79	0.64	2 : 12
S1 High	0.64	0.21	1 : 13
S2 Low	0.71	0.43	2 : 12
S2 High	1.07	11.32	5 : 9

Plants were assessed 340 days post inoculation.
$ Low = 10 ml of 3.3 x 10^6 spores/ml, high = 10 ml of 3.3 x 10^7 spores/ml..

There are no statistically significant differences between isolates ($P > 0.05$; chlorosis and % browning Kruskall Wallis, frequency of stem browning Chi-squared).

4.6. DISCUSSION

The immediate aim of this section was to investigate ways of improving current practices in nursery and field wilt trials at Binga.

Experiments with inoculated seedlings clearly demonstrated that severe and quicker symptom development could be induced by the application of a higher level of inoculum to younger plants. These results were in agreement with previous reports on the effects of inoculum level (Locke & Coulhoun, 1974) and plant age (Renard *et al.*, 1972).

Comparisons of disease assessment methods demonstrated that measurements of stem tissue browning (as used in African nursery trials) generally produced the most significant results and unlike the more time consuming measurements of growth or transpiration did not require any uninoculated control plants. These experiments also demonstrated that assessment of stunting could be improved by measuring the height of the youngest fully expanded leaf rather than total plant height.

By adopting the improvements suggested by experiments with seedling plants it was possible to greatly improve the efficiency of the clonal nursery trials (previously conducted at Plant Breeding International, Cambridge). However, there may be a tendency to reject any clone that is significantly more susceptible than a standard resistant line. In the present study this would have

resulted in the rejection of all other clones. Therefore this test procedure and the standard resistant clone should be calibrated against material of acceptable field resistance.

In some clones, a lack of correlation between different symptoms indicated that different disease characteristics may not be associated. It could be expected that the quantity of the pathogen in the plant would relate to host response (stem tissue browning) and pathogenic effects on host physiology (stunting). However, browning was a cumulative indicator of host response, cfus indicated the quantity of the pathogen at the time of harvest and stunting reflected the host physiology when the measured leaf was developing. As some of these characteristics are cumulative and some may not develop at the same rate it is not surprising that correlation was not always observed. Furthermore, it is possible that fungitoxic chemicals were released from bulb stem tissue during comminution for cfu analysis and would inhibit the development of fungal colonies. Such technical problems could mask correlation or produce correlations of their own. Nevertheless, all symptom assessment methods demonstrated that the standard resistant clone was the least affected by inoculation.

The use of more severe inoculation methods (root dip and cut root inoculation) greatly increased the speed and severity of symptom development. However, these techniques induced most symptoms in a cross that was previously demonstrated to be the most resistant in the soil

inoculation experiments. Therefore these techniques should be refined by further experimentation (perhaps by the use of a lower inoculum concentration) so that they speed up symptom development without rejection of potentially resistant material.

Prendergast (1963) similarly regarded root dip inoculation as too severe a test, but his observations may also reflect other factors associated with nursery trials in Africa. Thus, infected plants frequently die within 6 weeks of showing their first symptoms at Binga (Nyanduza, 1988, pers comm) while under UK glasshouse conditions such deaths are rare.

A possible cause of this premature plant death may be a combination of inoculation and the very high soil temperatures observed around plants in the nursery. However, experiments revealed that such elevated temperatures reduced the growth of the pathogen *in vitro*, and suppressed the development of symptoms in inoculated seedlings, while the growth of uninoculated palms was unaffected.

These results concur with previous reports that elevated root temperatures (34^{o}C) increased the resistance of a susceptible banana cultivar to *Fusarium* wilt (Beckman, Halmos & Mace, 1962), whilst raising temperatures (40^{o}C) can reduce the viability of *Fusarium* propagules (Freemann & Katan, 1988) and heating soils to 44-48 ^{o}C increased the suppressiveness of soils to *F.*

oxysporum f.sp *lycopersici* (Greenberger, Yogev & Katan, 1987). Therefore, the infection rate in nursery trials at Binga may be further improved by the readoption of shading, a practice that is still followed in the Ivory Coast (Renard, 1991, pers comm).

In conclusion, the results from UK glasshouse experiments suggested that nursery trials in Zaire could be improved by the adoption of a standard isolate applied at a higher dose to younger plants, and that each trial should include standard resistant and susceptible material. Although the measurement of stem browning was found to be the most efficient disease assessment method it requires destructive sampling. Ideally, a trial should be standardised against disease development rather than a fixed time. Therefore a whole trial could be assessed when a standard cross had reached a predetermined level of stem tissue browning. This could be determined by destructive subsampling of plants at regular intervals. Alternatively nondestructive observations of chlorosis or stunting (which this study has shown to correlate to stem browning) could be used to predict when final harvesting should occur. If trials could be standardised on a set level of disease development then wilt indices, as used by Renard *et al* (1972), which compensate for differences in symptom development between trials, would no longer be required.

Some, but not all, of the improvements described above have been adopted at Binga. The use of standardised inoculum and standard resistant and susceptible material

for each trial has been accepted, but there has been a natural reluctance to abandon techniques that have produced adequate results in the past (Rosenquist, 1988, pers comm). Thus the trials continue to be conducted with a large number of plants (*ca.* 50 per cross) which are destructively harvested after a set time. Experiments with the use of higher levels of inoculum, shading and growth measurements of inoculated and uninoculated plants have been conducted in Zaire.

Although the use of high dose inoculation at Binga increased symptom development (by between 18 to 100%), the results from low dose inoculation were regarded as being sufficiently similar as to make the increased effort involved in producing high concentrations of inoculum unjustifiable. The recommended use of shading decreased the level of infection, but this experiment was conducted during the rainy season, when the frequency of high soil temperatures may have been reduced and will be repeated during the dry season. Growth measurements from inoculated and uninoculated palms from Binga are not yet available.

The increased infection rates (from 30 to 60% for susceptible crosses, Van Amstell, 1990, pers comm) in nursery trials in Zaire is probably mainly due to the use of a standard inoculum. Thus, while adoption of some of the above recommendations may improve results under UK conditions, in Zaire, acceptable results for seedlings can be obtained with a low level of inoculum, destructive assessment and a high level of replication.

However, for logistical reasons it is difficult to conduct regular, highly replicated trials with clonal material in Zaire. Using the improved techniques for glasshouse experiments it is now possible to conduct such trials in the UK, and > 30 different clones have or are now being screened here on a commercial basis.

The increment borer proved an excellent tool for the assessment of disease in field palms; it effectively removed cores from palm trunks, allowing observation of brown vascular bundles which are diagnostic for infection by Foe. Generally, this limited survey (70 palms) was in close agreement with the wilt survey (vascular browning was observed in 90% of palms regarded as wilted in the census) and confirmed that the census, which requires less time and effort, provided a reasonably accurate estimate of disease.

However, stem vascular browning was also observed in > 25% of palms regarded as healthy in the census, which indicates that core sampling was a more sensitive indicator of infection.

A more extensive survey of field palms is planned, partly to confirm the above results, but also to demonstrate the frequency of infected yet symptomless palms in the plantation. In a large survey of the effect of agronomic practices on *Fusarium* wilt, Renard and de Franqueville (1991) reported that 8% of 6 yr old symptomless palms contained brown fibres in the trunk and

that there was a 3 to 15% yield reduction in these palms. They also observed brown fibres in the trunk of 50% of older palms with no obvious symptoms of wilt. In another report (Renard & de Franqueville, 1990) they also observed a low level of infection from which the palms later recovered, in 16 to 13 % of 11 to 14 yr old palms (here infection was only assessed by foliar symptoms).

This last report did not indicate if such latent infection was more common in some crosses than others, but results from core sampling at Binga did show that some crosses may be totally immune while others had a high level (50%) of latent infection. Therefore, breeding programmes based on obvious external symptoms alone may produce tolerant rather than resistant material. In conclusion, results from this study and and by Renard & de Franqueville (1990, 1991) suggest that infected symptomless palms may be common in plantations and could result in significant yield reductions.

Microscopic examination of cores from trunks and root samples enabled, for the first time, quantification of the level of vascular occlusion in different parts of the palm without destruction of the tree. There was a highly significant correlation between the level of vascular occlusion and leaf stunting (3.3.9.), which confirmed that examination of cores provided an accurate estimate of disease severity.

In diseased palms, a high level (nearly 50%) of vascular occlusion was observed in the trunk, which contrasted with a negligible incidence of occlusion in root vessels. These findings concur with earlier observations (Wardlaw, 1950; Prendergast, 1957) that even in severely diseased palms, most roots appeared to be healthy, and only a few roots contained brown tissue in the stele. These authors did not examine roots microscopically or attempt reisolation of the pathogen, but presumed that a brown or black root core indicated infection by Foe. However, they were able to excavate entire stem bases and sampled many more roots than was possible in this investigation. This may partly explain why they found diseased roots in infected palms, while in this study no diseased roots were detected.

The low incidence of root infection led Wardlaw (1950) to suggest that stem colonisation could follow infection of only a few roots, or that the pathogen could enter the stem through the fissures in the central base of the trunk. These fissures are caused by internal expansion during establishment growth of the trunk (Tomlinson, 1990).

Alternatively, the discrepancy in root and trunk xylem colonisation could reflect differences in the permanence of the xylem system in these 2 regions of the palm. Due to a lack of secondary thickening, palm trunk vascular tissue is irreplaceable (Zimmerman & Brown, 1971) and therefore the level of vascular occlusion in the trunk would reflect

the cumulative infection over the life of the palm. In contrast Dufrene, Ochs and Sangier (1990) estimated that the annual replacement rate of primary, secondary and tertiary roots in oil palm was 15, 31 and 57% respectively, so cumulative infection of the replaceable root xylem system is less likely. However, even if the replacement rate of roots suggested by Dufrene *et al* (1990) is correct it is unlikely to fully account for the considerable discrepancy between root and trunk vascular colonisation observed in 6 yr old palms in this study. Indeed, Tomlinson (1990) suggested that there was no evidence for the replacement of palm roots and that they could be regarded as permanent structures..

Alternatively, architectural differences between the xylem system in roots and stem may explain the discrepancy in vascular occlusion between these areas. Hence, in palm stems the xylem system is highly interconnected (Zimmerman & Brown, 1971), while there is obviously no vascular interconnection between roots and microscopic examination has indicated vascular bundles in roots are discrete. This suggests that systemic colonisation of the xylem system would be much easier in the stem than the roots.

Therefore a critical feature of palm invasion appears to be colonisation of the stem, which could follow infection of only a few roots. Locke and Colhoun (1977) demonstrated that palm seedlings could become infected through pneumathodes on roots. However it is also possible

that infection could occur directly into the stem, either through stem fissures (Wardlaw, 1950) or at the point where new roots erupt from the stem base. These points obviously require further investigation.

Isolates of the pathogen from diseased nursery and field palms of resistant clone 28 were shown to be no more aggressive or virulent than the standard Binga isolate. This indicated that clone 28 had not, as yet, selected out new forms of the pathogen, as occurred with field plantings of tomato (Gerdemann and Finley, 1951). Here plants resistant to race 1 were colonised by race 2 of the pathogen, which was presumed to have arisen from race 1 by mutation. However, Pegg (1974) suggested that such apparent *de novo* appearances of host specific strains of a pathogen were probably due to selection from the population of soil strains rather than adaptation. However, with modern techniques the origin of new races of pathogens can be studied in much greater detail. Thus, analysis of vegetative compatibility groups has shown that race 4 of *F. oxysporum* f.sp. *cubense* was not introduced into the Philippines on contaminated material imported from Taiwan, as had previously been presumed (Ploetz, 1990).

Genetic variation within an oil palm clone is believed to be uncommon (< 5%, based on measurements of physiology and growth, Eeuwens, 1991, pers comm). Therefore the development of disease in some palms of the resistant clone 28 support the theory of the threshold nature of

disease resistance (Renard *et al.*, 1972) rather than any genetic differences in host or pathogen.

However, the threshold nature of disease resistance may also reflect the qualitative nature of disease assessment; any seedling without brown stem tissue or field palm without leaf symptoms are presumed to be uninfected. In field palms this presumption can be incorrect, and in this study up to 50% of apparently healthy palms in one cross were shown to be infected. This result may indicate that core sampling can reveal infection before leaf symptoms become apparent, or that infected symptomless palms are tolerant. Continued observation of these trees is required. In contrast, the total absence of trunk colonisation in some other crosses, particularly when the palms had been in the field for many years, suggested that the plants were resistant rather than tolerant, and confirmed earlier reports of total field resistance in some crosses (de Franqueville & de Greef, 1987; Rosenquist, 1988, pers comm)

Currently resistant seed and clonal material is produced from palms with no obvious disease symptoms from progenies with a lower than average incidence of wilt. This procedure presumes that the resistance/tolerance within a particular progeny is expressed equally in all palms of that cross (Renard *et al.*, 1972). However, a recent study (de Franqueville *et al.*, 1991) demonstrated large differences in resistance between individual trees within a progeny. This inconsistency combined with the

variability of plantation conditions means that an apparently healthy field palm of a cross of low disease incidence cannot be presumed to be highly tolerant. The risk of propagating seed or clones from susceptible yet apparently healthy palms can be reduced by examination of trunk core samples, and this procedure has now been adopted at Binga. Ultimately a test is required whereby the resistance of individual field palms can be assessed, such a model system is currently being developed in this laboratory.

As discussed previously (4.1) theories on the inheritance of resistance remain confused. There is evidence to suggest that resistance is inherited through the action of many genes (Menuier *et al.*, 1979) or a few (de Franqueville & de Greef, 1987). These genes may confer resistance that segregates (de Franqueville *et al.*, 1991) or is expressed evenly (Renard *et al.*, 1972) throughout the palms of a particular cross. Furthermore some reports suggest that differential isolate-cross interactions are also possible (Aberungboye, 1981; Oristsejafor, 1989; Renard, 1991, pers comm).

In part this confusion may reflect the difficulty of conducting trials on a perennial crop in Africa involving the assessment of a disease which is strongly influenced by environmental factors and where resistance is not usually expressed as immunity to infection. Under such conditions, the interpretation of percentage infection

figures as evidence for the segregation of resistance genes is very difficult.

Another problem may be a lack of genetic diversity within this crop where a restricted gene pool has been repeatedly inbred (Hardon, 1970; Porter, 1989) and where selection for wilt resistance is restricted to material already collected on the basis of yield potential rather than expanding genetic collections in search of greater resistance (Porter, 1989).

A better understanding of the mechanisms underlying host resistance and susceptibility is required and may be obtained by concentrating on the extremes of host response. In particular, crosses that demonstrate total field resistance should be examined in greater depth, and seed should be collected from healthy palms in the semi-natural groves in West Africa. For either of these selections it is important to distinguish between tolerant and resistant palms. Seeds (preferably self pollinated) should only be collected from old symptomless trees which have been sampled with the increment borer.

The exploitation of resistance from grove palms, which may well be associated with agronomically poor characteristics, would probably be impossible if resistance were inherited in a polygenic manner, but may be possible if monogenic or oligogenic mechanisms were involved (Vanderplank, 1982). However, such resistance may be vulnerable (particularly in a perennial crop) to the

development of virulent isolates of the pathogen, as has occurred with *Fusarium* wilt of tomato (Gerdemann & Finley, 1951) banana (Buddenhagen, 1990) and peas (Goth & Webb, 1981).

Therefore, the breeding programme at IRHO which is believed to select for polygenic additive resistance may ultimately produce a stable resistance. However, polygenic additive resistance could be confused with oligogenic resistance based on just a few genes and although polygenic resistance is usually more stable than resistance based on one or a few genes (Day, 1974; Vanderplank, 1982, 1984), single gene resistance has proved effective in other vascular diseases, such as *Fusarium* wilt of cabbage and tomato. Thus, although isolates of *F. oxysporum* f. sp. *cubense* that are pathogenic to the otherwise resistant Cavendish clones of banana do exist, this clone has remained resistant (for *ca.* 30 yrs) in the Americas and tropical Africa (Buddenhagen, 1990). In this case the number of genes for resistance is unknown but Buddenhagen (1990) concluded that such vertical (*sensu* Vanderplank, 1984) resistance to monocyclic soil-borne diseases was usually durable and stable. However, as discussed in the next chapter (5), there is strong evidence that in *Fusarium* wilt of oil palm the pathogen is air-borne and sporulates profusely on male inflorescences, and thus it cannot be regarded as a typical soil-borne, moncyclic disease.

Obviously much work on the nature and inheritance of disease resistance is still required. The development of reliable procedures for the infection and assessment of disease in clonal palms in the UK means it is now possible to conduct experiments on resistance mechanisms using clonal material of known resistance or susceptibility.

With this material it may be possible to develop a model system which can quickly distinguish between resistant and susceptible plants, and thus replace the time consuming pathogenicity test. Conversely, this technique may also allow a comparison of virulence of a range of isolates from around the world. Both these possibilities (the latter of which should only be conducted in an area where oil palm is not grown) are now being investigated at Bath.

5.0. EPIDEMIOLOGY OF *FUSARIUM* WILT OF OIL PALM

5.1. INTRODUCTION.

5.1.a. World-Wide Distribution and Spread

Oil palm probably originates from West Africa (Hartley, 1988) and until recently, although the crop is now grown throughout the tropics, this was the only area from which *Fusarium* wilt had been reported. The reasons for this restriction in disease occurrence are unclear since large quantities of seed have been exported from West Africa, and pathogenic isolates of *F.oxysporum* have been isolated from the shell (Locke & Colhoun, 1973) and kernel surface of seeds (Flood, Mepsted & Cooper, 1990).

Colhoun (1981) suggested that the disease may not have become established in some other areas, such as South East Asia, because they lacked the prolonged dry season which occurs in West Africa but Turner (1981) noted that wilt did not occur in China where the crop was exposed to prolonged drought. Alternatively, oil palm grown outside Africa (which has a comparatively narrow genetic base) could be resistant to the disease but Ho, Varghese & Taylor (1985a) found that Malaysian oil palms were very susceptible to wilt. Also, Malaysian strains of *F. oxysporum* (isolated from plantation soil and palms) were non pathogenic, although they can colonise roots (Ho *et al.*, 1985a) and stems (Flood *et al.*, 1989) of inoculated plants. Ho *et al* (1985a) concluded that the non-appearance of the disease in Malaysia was due to the absence of the

pathogen, and that strict quarantine measures were needed to prevent its importation on contaminated material from Africa.

However, Rosenquist (pers comm, 1989) suggested that there was no serious risk of spreading the disease by this method, as experience had shown that despite the importation (for > 40 yrs) of large quantities of potentially contaminated seed from Africa, Malaysia had remained wilt free.

Similarly, Snyder (cited by Baker, 1981) reported that despite the importation of large quantities of contaminated pea seed and extensive cultivation of susceptible varieties, *Fusarium* wilt of pea only occurred in 2 areas in California. This failure of the disease to spread was found to be due to the suppressive nature of California soils. Similar soil suppressiveness has been reported in other vascular wilt diseases (Baker, 1981; Louvet, Alabouvette & Rouxel, 1981), thus the absence of wilt in Malaysia may be due to its soils being suppressive to the establishment of Foe.

In contrast to S.E. Asia, there have been reports of wilt in Brazil (Van de Lande, 1984) and Ecuador (Renard & de Franqueville, 1989), and the pathogenicity of isolates from these locations was confirmed by inoculation of young palms (Flood Cooper & Lees 1989; Paul, pers comm 1992). Furthermore, experiments on vegetative compatibility as determined by nitrate non-utilising (nit) mutants (Flood,

Whitehead & Cooper, 1992), and by RFLP analysis (Mouyna, 1992) has demonstrated that these isolates from S. America were compatible with and had identical RFLP banding patterns to those from the Ivory Coast. S. American isolates were not compatible with isolates from Nigeria or Zaire. These results suggest that the Ivory Coast and S. American isolates have a common origin, and indeed the affected plantations in S. America were planted with seed imported from the Ivory Coast. It is therefore probable that the disease was introduced to South America on contaminated seed from this source.

5.1.b. Occurrence and Spread Within Infected Areas

Within a plantation, wilt frequently occurs on palms adjacent to previously infected plants and high levels of infection have been observed when areas of old palms have been replanted, particularly if new palms were planted adjacent to the stumps of old trees (Prendergast, 1957; Renard & Quillec, 1983; Renard & de Franqueville, 1989).

These results suggest that the population of pathogenic *F. oxysporum* in soil may increase in the presence of the host. However, quantification of populations of Foe are difficult due to the problem of distinguishing between pathogenic and non-pathogenic isolates. Locke (1972) concluded that all 22 soil isolates he tested were pathogenic since they could be reisolated from surface sterilized roots of inoculated seedlings. However, Renard (1967; pers comm, 1991) reported that very few (< 1 per 1000) soil isolates of *F. oxysporum* could induce symptoms

of leaf chlorosis, stunting or vascular browning in inoculated seedlings and concluded that the vast majority of soil isolates were non-pathogenic. Furthermore, preinoculation of seedlings with non-pathogenic isolates of *F. oxysporum* from Africa significantly reduced the level of infection (to 20-50% of controls) in inoculated seedlings (Renard, 1976; Taquet, 1985).

Pathogenic isolates of *F. oxysporum* (based on symptom development rather than reisolation) have also been found in some weed species in African plantations (Oritsejafor, 1986) and, significantly, in air samples (Cooper, Flood & Mepsted, 1989). The latter authors suggested aerial dispersal could have important implications for the rate of spread of what had previously been regarded as a soil or seed-borne disease. They could find no evidence of systemic colonization of male or female inflorescences in infected palms, and reported similar levels of external contamination on inflorescences from uninfected palms. Therefore, they concluded that seed and pollen contamination by *F. oxysporum* was probably from an external source(s).

The experiments described in this section investigate the pathogenicity of isolates of *F. oxysporum* from plantation soils, pollen samples and male inflorescences; also the contamination of seed and pollen and possible protocols for their decontamination are investigated. The role of non-pathogenic Malaysian isolates of *F.oxysporum*

in preventing the establishment of this disease in Malaysia is also examined.

5.2. RESULTS.

5.2.1.a. Quantification and Pathogenicity of Soil Isolates of *F. oxysporum*.

Depending on the methods and definitions used, soil isolates of *F. oxysporum* may be pathogenic (Locke, 1972) or non-pathogenic (Renard, 1967; pers comm, 1991). One possible explanation for this apparent discrepancy is that nearly all the isolates tested by Locke were from Zaire while Renard used Ivory Coast isolates. Thus, an experiment was performed to further investigate the pathogenicity of soil isolates from Binga, Zaire.

Soil samples, taken 10mm below the surface, were removed from the base and 2m from the trunk of apparently healthy trees in an area where *ca.* 10% of palms were wilted. One g (wet weight) was suspended in 9ml of sterile distiled water, a dilution series was prepared and 1ml of each concentration was plated onto *Fusarium*-selective medium (Appendix 1). The remainder of the sample was weighed, oven dried (60^{o}C for 24h), reweighed and the quantity of *Fusarium spp.* was calculated as colony forming units (cfus) per g dry weight of soil (Table 54).

Both *F. oxysporum* and *F. solani* were present at similarly high levels (> 1 x 10^4 cfus g^{-1}) in the soil and *F. oxysporum* occurred at higher levels 2m from the palm than at the stem base.

Table 54: Propagules of *Fusarium spp* in Plantation Soil.

	Inoculum Level *	
	Base of Trunk	2 Metres from Trunk
F. oxysporum	10.6	19.0
F. solani	15.9	14.9

* Colony forming units per g dry weight of soil x 10^3. Values are the mean of 7 replicates. There are no significant differences between values ($P > 0.05$, Mann Whitney U-test).

The pathogenicity of 4 of the *F. oxysporum* isolates (2 each from the stem base and 2m from the palm) was tested by inoculation of susceptible UF4 clonal palms at low dose (2.2). Symptom development was assessed by height measurements, reisolation from the stem and internal symptoms (2.3.1a, 2.3.2b, 2.3.3) and compared with that produced by a pathogenic isolate (F3) (Table 55).

None of the soil isolates induced any disease symptoms (although *F. oxysporum* was reisolated from the stem of one inoculated plant) and therefore the soil isolates tested were regarded as non-pathogenic.

Table 55: Pathogenicity of Soil Isolates of *F. oxysporum*.

	Height of Palm (mm)	Percentage Browning of bulb stem tissue	Ratio of Palms With:Without Reisolation of the Pathogen from the Stem Base
Control	798a	0	0:12a
F3 *	474b	51.4	9:3b
Soil 1	784a	0	0:12a
Soil 2	791a	0	0:12a
Soil 3	789a	0	0:12a
Soil 4	760a	0	1:11a

Values are the mean of 12 replicates, 365 days post inoculation.
* Isolate of known pathogenicity.
Within each column, figures with the same letter are not significantly different (P> 0.05, height data :- Kruskall Wallis and repeated Mann Whitney U-test, Reisolation:- Chi-squared and Fisher's Exact test).

5.2.1.b. Quantification and Pathogenicity of Isolates of *F. oxysporum* from Male Inflorescences.

Male inflorescences of oil palm liberate pollen for about 5 days after anthesis before becoming covered with mycelium, of which *Fusarium* is the most frequently encountered genus (Turner, 1981). The size and abundance of these old fungus-covered inflorescences (each consisting of 100-300 spikelets, measuring 100-200 mm in length and 8-15 mm in diameter, and frequently >4 per palm) suggested that they could be a significant source of *F. oxysporum* inoculum. The quantity and pathogenicity of *F. oxysporum* from this source was therefore investigated.

Seven male inflorescences were collected at random within the plantation, each was washed in 2 l of sterile distilled water and a dilution series prepared from the resultant spore suspension. One ml aliquots of this suspension were plated onto *Fusarium* selective medium (see Appendix 1) and the number of *Fusarium spp* propagules per inflorescence was determined.

Both *F. oxysporum* and *F. solani* were present at high levels (mean values respectively 7.2 & 50.0 $x10^6$ cfus per inflorescence), and in contrast to soil samples *F. solani* was present in significantly higher levels than *F. oxysporum* ($P < 0.05$, Mann Whitney U-Test).

The pathogenicity of 2 of these isolates was tested (by J. Flood) by inoculation of UF4 clonal palms, as in the previous section. Symptom development was assessed by use of a wilt index (Flood, Cooper & Lees, 1989) and dry weight of root and aerial tissue (Table 56). Both isolates induced significant levels of symptoms and were therefore regarded as pathogenic.

Table 56: Pathogenicity of *F. oxysporum* Isolates from Old Male Inflorescences and Pollen Samples.

	Wilt Index	Dry Weight (g)	
		Roots	Aerial Tissue
Control	0.1a	19.5a	40.3a
F3 *	3.0b	6.9b	20.4b
Inflorescence 1	2.8b	6.1b	13.9b
Inflorescence 2	1.8b	10.5b	22.9b
Pollen 1	2.0b	8.9b	20.7b
Pollen 2	2.1b	8.9b	16.8b

Values are the mean of 12 replicates UF4 clonal plants, 308 days post inoculation.
* Isolate of known pathogenicity
Within each column, figures with the same letter are not significantly different (P> 0.05, Wilt Index :- Kruskall Wallis and repeated Mann Whitney U-test, Dry weight :- Anova and Bonferroni's test.).

5.2.1.c. Quantification and Pathogenicity of Isolates of *F. oxysporum* from Pollen Samples.

Although *F. oxysporum* had previously been isolated from a pollen sample (Flood, pers comm, 1989), the frequency of this contamination and the pathogenicity of isolates from this source were unknown.

Contamination levels of *Fusarium spp* in 30 samples of freeze dried pollen were estimated by a serial dilution of 0.03g of pollen in sterile distilled water, and plating onto *Fusarium*-selective medium (see Appendix 1). After 4 days incubation (at 28^{o}C) *Fusarium* colonies were counted and the number of cfus per g of pollen was determined.

F.solani was present at high levels and was isolated from 27 samples at levels up to 2.3 x 10^6 cfus g^{-1}, while *F. oxysporum* was obtained from 50% of samples at much lower levels (up to 3.6 x 10^5 cfus g^{-1}).

The pathogenicity of 2 pollen isolates to UF4 clonal palms was assessed, as in the previous section; both isolates induced significant levels of wilt symptoms (Table 56) and were therefore regarded as pathogenic.

5.2.2.Decontamination of Seed and Pollen.

Reports of the contamination with Foe of the shell (Locke & Colohoun, 1973) and kernal surface (Flood *et al.*, 1990) of seeds and of pollen samples (5.2.1.c.,above), have resulted in Malaysia placing restrictions on the importation of such material from Africa. However a prolonged ban would restrict breeding programmes in Malaysia, where the genetic base of the crop is relatively narrow (Hartley, 1988). Therefore, to facilitate international breeding programmes and reduce the risk of further disease spread (as has occurred in S. America) techniques were required for the decontamination of seed and pollen.

5.2.2.a. In vitro Selection of Fungicides.

Seed produced at Binga, Zaire is routinely dusted with Thiram and DDT, but this treatment does not eradicate pathogenic *F. oxysporum* from the seed shell or kernel surface, in fact more contamination was reported on treated seed (Flood *et al.*, 1990).

Initially a range of fungicides (Benomyl, Captafol, Chlorothalonil, Prochloraz, Imazalil & Iprodione; see Appendix 4 for suppliers) were screened for their effect on the germination of Foe microconidia *in vitro*. These fungicides were selected either on the basis of their ability to suppress mycelial growth of Foe *in vitro* (Kendall, 1985), or for their known activity against *Fusarium* spp *in vivo* (Anon, 1991).

Each fungicide was mixed with sterile distilled water (SDW) to produce concentrations from .01mg to 1g a.i. l^{-1}. Twenty μl of each concentration was mixed with 15 μl of a spore suspension (10^5 microconidia ml^{-1}) on a coverslip in a humid chamber at 25 ^{o}C, and after 24h spore germination was assessed.

Chlorothalonil and Captafol were by far the most active fungicides tested and prevented germination at 0.1 & 0.01g a.i l^{-1} respectively.

5.2.2.b. Fungicide Decontamination of Naturally Contaminated Seeds.

Fungicides which prevent microconidia germination *in vitro* may not effectively decontaminate seeds, where the fungus may exist as chlamydospores. Therefore, fungicides used in the previous section were applied to naturally contaminated tenera seed (cross 312/3 x 312/3).

However, elimination of Foe on the kernel surface will not occur if fungicide is applied to the outside on the seed alone, the fungicide has to penetrate inside the seed

i.e. to the space between the shell and kernel. To achieve this, seeds (15 per treatment) were submerged in 100 ml of SDW containing fungicide (1g a.i. l^{-1}) and exposed to a partial vacuum (*ca.* 700 mm Hg) for 1 minute. Under these conditions, air was drawn out from between the shell and kernel, into which water infiltrated when the vacuum was released (as evidenced by a wet kernel surface if the seed was cracked open). This procedure was repeated 3 times. There were 2 control treatments, one in which fungicide was applied without vacuum infiltration and another with infiltration of SDW. Treated seeds were then air dried for 2 days, aseptically cracked and the kernels removed. Kernels were placed in 10ml of SDW, agitated for 12s, left for 10 min then shaken again, before a dilution series was prepared. One ml of each dilution was plated onto *Fusarium*-selective medium and incubated as previously described (see Appendix 1 & 2.3.3).

F. oxysporum was not isolated from any kernels, whilst *F. solani* was frequently detected. Previous experiments (Mepsted, unpublished) indicated that *F. solani* usually occurred on seeds at *ca.* 10 times the level of *F. oxysporum*; therefore the failure to detect *F. oxysporum* may be because it was totally masked by *F. solani*. After 4 days incubation, 64% of fungicide-treated control (non-vacuum infiltrated) seeds showed contamination by *F. solani*, (mean 1,674 cfus per kernel); as did 43% of control seeds infiltrated with water (mean of only 69 cfus per kernel). However, this apparently marked difference in

cfus per kernel was mostly due to a few highly contaminated seeds in the first treatment and was not statistically significant ($P > 0.05$, Mann Whitney U-Test).

At this stage, no *Fusarium* sp. or any other fungus had grown from the Captafol- or Chlorothalonil-treated seeds, while all other fungicides produced results similar to the controls. However, after 7 days incubation small colonies of *F. solani* were visible on the Captafol- and Chlorothalonil-treated plates; 20% of kernels were still infested (mean 25 cfus) after Captafol treatment and following Chlorothalonil treatment, 27% were contaminated (mean 28 cfus).

5.2.2.c. Decontamination of Artificially Infected Seeds.

Although vacuum infiltration with Captafol reduced contamination by *F. solani*, further work required a reliable supply of seeds infested with *F. oxysporum*. Yet previous observations (Mepsted, unpublished) had indicated that natural contamination of seed with *F. oxysporum* was often low and perhaps easily masked by *F. solani*. To overcome these problems, seeds were artificially infested with *F.oxysporum*. An early attempt to do this in this laboratory was unsuccessful, apparently due to the diversity and quantity of microflora already present on the seeds. Therefore, during a visit to Zaire, freshly harvested seeds were infested by the following procedure.

One hundred ml of a 5 day spore suspension (see 2.2) of Foe were added to *ca.* 1000 freshly depericarped tenera seeds (H3/27, code 108) in a large plastic bag. The

inoculum and seeds were mixed and incubated at room temperature for 24h, excess fluid was drained off and the seeds were incubated for a further 4 days. Seeds were then air dried, placed in clean plastic bags and dispatched to Bath.

Seeds were then treated following the standard germination procedure for commercial seeds (Hartley, 1988), *i.e.* soaked for 1 week (with daily changes of water), heat treated (38^{o}C for 75 days), soaked for another week and then germinated at 25^{o}C.

Captafol, the most active fungicide in previous experiments, was applied (1g a.i. l^{-1}) during the first soak period using one of 2 techniques:- 1) vacuum infiltration with fungicide, as in the previous section but on this occasion, followed by a soak for 7 days with daily changes of fungicide; or 2) no vacuum treatment but soak for 7 days (with daily changes of fungicide). Control seeds were soaked in water. After soaking, seeds were air dried and the mean number of cfus of *Fusarium* spp were determined, as in the previous section (Table 57a,b).

F. oxysporum and *F. solani* were found on the shell and kernel of all seeds not treated with fungicide, and despite deliberate infestation with *F. oxysporum*, *F. solani* was still present in higher levels. On seeds soaked in Captafol, *Fusarium* spp were eradicated from the shell (external surface) but *F. solani* was found on all kernels and *F. oxysporum* on 13 out of 15 kernels. However, vacuum

infiltration with Captafol, completely eradicated *Fusarium* spp from both the shell and kernel.

Table 57a: Effect of Fungicide Treatments on the Level of *F. oxysporum* on Seeds.

	Mean Colony Forming Units Per	
	Shell*	Kernel
Control (water soak)	3402	274
Captafol (soak)	0	155
Captafol (vacuum infiltration and soak)	0	0

Table 57.b.: Effect of Fungicide Treatments on the Level of *F. solani* on Seeds.

	Mean Colony Forming Units Per	
	Shell*	Kernel
Control (water soak)	4040	1068
Captafol (soak)	0	1458
Captafol (vacuum infiltration and soak)	0	0

*Shell = shell external surface.
Values are the mean of 20 replicate seeds pre heat treatment.

Vacuum infiltration could conveniently be applied to seeds when they are soaked prior to heat treatment (to break dormancy) as above; or at the post heat treatment soak (which induces germination). This latter application may be more effective since seeds would be delivered to the customer soon after treatment, thus reducing the risk of reinfestation. The previous experiment was therefore repeated with the batch of seeds after they had been heat-treated.

In this experiment, the replication was increased (60 seeds per treatment) and infection was assessed as cfus per shell/kernel, as before, or by direct plating of shells or kernels onto selective medium. Direct plating was adopted because it prevented any decrease in fungicide concentration that probably occurred during the preparation of a dilution series, and yet demonstrated if some areas of the kernel surface had not been exposed to fungicide during treatment.

Heat treatment reduced the levels of both *F.oxysporum* and *F. solani* in seeds not treated with fungicide, but these fungi were still present at significant levels in a large number of seeds (Tables 57, 58 & 59). However, vacuum infiltration with Captafol eradicated all *Fusarium* spp from both the shell and kernel.

Table 58: Effect of Captafol on the Levels of *Fusarium spp* on Heat Treated Seeds.

	Colony Forming Units of *F.oxysporum* per		Colony Forming Units of *F. solani* per	
	Shell	Kernel	Shell	Kernel
Control *	40	7	127	200
Captafol *	0	0	0	0

Values are the mean of 60 replicates.
* Control = Heat treatment (75 days at 39°C) followed by soaking in water for 7 days (change water daily) at 25°C.
Captafol = As control, but vacuum infiltrated with Captafol after heat treatment and 7 day soak in Captafol.

Table 59: Effect of Captafol on the Presence of *Fusarium spp* on Heat Treated Seeds.

	Ratio of With:Without Reisolation of *F. oxysporum* from		Ratio of With:Without Reisolation of *F. solani* from	
	Shell	Kernel	Shell	Kernel
Control *	34:26	18:40	55:5	2:56
Captafol *	0:60	0:58	0:60	0:58

Values are the mean of 60 replicates.
* Control = Heat treatment (75 days at 39°C) followed by soaking in water for 7 days (change water daily) at 25°C.
Captafol = As control, but vacuum infiltrated with Captafol after heat treatment and 7 day soak in Captafol.

5.2.2.d.Effect of Fungicide Treatment on Seed Germination and Plant Development.

Although vacuum infiltration with Captafol eradicated Foe from seeds, the phytotoxicity of this treatment had yet to be investigated. Its effect on seed germination was determined by comparing the germination rate of

artificially inoculated seeds which had been heat treated and vacuum infiltrated with Captafol (as above), with similarly treated seeds that had received no fungicide. Germination was determined after incubation in plastic bags at 25°C for 3 weeks. Germinating seeds were then propagated (2.1) to assess the effect of fungicide treatment on plant growth and development.

The germination of fungicide-treated seeds was similar to controls (respectively 91 & 79 out of 110), and after one month in the glasshouse, no growth abnormalities were observed in any seedlings.

5.2.2.e. Seed Transmission of *Fusarium* Wilt.

Although pathogenic isolates of *F. oxysporum* have been obtained from the shell (Locke & Colhoun, 1973) and kernel surface (Flood *et al.*, 1990) of palm seeds, it remained to be established if infected plants could be produced from infested seed.

To investigate this possibility, sixty fungicide treated and untreated seedlings from the above experiment were maintained in the glasshouse for another 8 months. Symptom development and infection was assessed by a wilt index (Flood *et al.*, 1989), by reisolation from the stem and from internal symptoms (2.3.3 & 2.3.2.b).

Plants grown from Captafol treated seeds had no internal or external disease symptoms and *F.oxysporum* was not reisolated from any of these palms. However, 3 plants grown from untreated seeds developed external symptoms of

wilt and 2 of these palms contained characteristic brown stem tissue from which *F. oxysporum* was reisolated.

In summary, a range of fungicides were assessed for their ability to eradicate Foe *in vitro* and, by vacuum infiltration, on naturally infested seeds. The most effective fungicide (Captafol) completely eradicated Foe from artificially infested seeds, and a small proportion of infested seeds not treated with fungicide gave rise to infected plants.

While soil isolates of *F. oxysporum* were shown to be non-pathogenic, those from male inflorescences and pollen samples were pathogenic and half the pollen samples examined were contaminated with *F. oxysporum*. A method for the decontamination of pollen was therefore still required.

5.2.2.f. Decontamination of Pollen.

To efficiently treat pollen with fungicide the chemical should be evenly deposited onto pollen grains. Such incorporation would be facilitated by the application of fungicide in a liquid but water, the conventional fluid for this purpose, would induce pollen germination. However, Mishra & Shivanna (1985) and Jain & Shivanna (1988) reported the successful storage of pollen in organic solvents. Accordingly, it was possible that fungicide could be delivered with an organic solvent without affecting pollen viability. Also some solvents may be fungitoxic on their own. Therefore, the toxicity of

various solvents to *F. oxysporum* on pollen and their effect on pollen viability were examined.

Eight solvents (propanol-2-ol, diethyl ether, acetone, chloroform, toluene, carbon tetrachloride, ethyl acetate & ethanol) were investigated by placing 0.03g of pollen in 5ml of solvent for 2h. The pollen was then separated by filtration (through Whatman No3 filter paper), air dried and streaked onto pollen germination agar (PGA) (Appendix 1). After 15h, pollen germination was assessed, and after 48h, plates were examined for the growth of *Fusarium* spp. A control treatment of pollen directly streaked onto PGA was also performed.

Pollen germination rates were high (*ca.* 70%) following treatment in all solvents, except ethanol in which no germination occurred. However, after 48h *F. solani* had grown from all pollen samples, although very weak growth was observed from pollen treated with diethyl ether.

It was considered that this solvent may have some antifungal activity, which may be used in conjunction with fungicides. Consequently, an experiment was performed in which diethyl ether was used as a carrier for Captafol or Daconil, (the fungicides most effective in eradicating *Fusarium* spp on seeds, 5.2.2.b.).

In this experiment, 0.01g samples of pollen were agitated in 5ml of diethyl ether containing a range of fungicide concentrations (from 5 to 0.005g a.i. l^{-1}) and left for 2h before filtration, as previously described.

Pollen viability was assessed by streaking onto pollen germination agar (Appendix 1), and the level of *Fusarium* was quantified by a serial dilution, in SDW, before plating onto *Fusarium*-selective medium (Appendix 1). Plates were incubated at 28°C, and pollen germination was assessed after 10 h and fungal growth after 3 days. There were 2 control treatments of diethyl ether or water without fungicide.

Both Chlorothalonil and Captafol prevented fungal growth but only at the highest concentration, (5g a.i. l^{-1}) (Table 60). However, at a Captafol concentration of 0.5g l^{-1}, although no fungal growth occured at the highest concentration of the dilution series, colonies of *Fusarium* spp grew on plates at ten and one hundred fold dilutions. This indicated that Captafol was not fungicidal to resting spores before germination occurred, and that it had been effectively removed from pollen samples by dilution in SDW.

Unfortunately, no pollen germination occurred in any treatment, including the controls nor in any of the other pollen samples then available. Therefore, at the time, no further experiments could be performed. Nonetheless, the technique had given promising results, and although fungicidal activity could be removed by dilution, this extent of dilution would not occur under normal conditions for pollen germination. However, for quarantine purposes, where total eradication of the pathogen should be guaranteed, this level of control may be insufficient.

Table 60: Effect of Fungicide Treatment on Pollen Contamination by *Fusarium spp.*

Treatment	Colony Forming Units per g of Pollen (x 10^3)	
	F. oxysporum	*F. solani*
Water	80	1350
Diethyl ether	90	1659
Captafol (5g a.i./l)	0	0
Captafol (0.5g a.i./l)	9	139
Captafol (0.05g a.i./l)	4	183
Captafol (0.005g a.i./l)	11	220
Chlorothalonil (5g a.i./l)	0	0
Chlorothalonil (0.5g a.i./l)	150	950
Chlorothalonil (0.05g a.i./l)	60	1650
Chlorothalonil (0.005g a.i./l)	30	2090

Values are the mean of 2 replicate plates.
Fungicides were applied in Diethyl ether.

Nonetheless, as previously discussed (5.1.a.), this disease has not become established in S.E. Asia despite the importation of large quantities of presumably contaminated seed and pollen from Africa. The cause(s) for this are unclear, especially when one considers that the disease has spread to S. America, but may involve some form of soil suppression, and consequently this possibility was investigated.

5.2.3. Cross Protection of Oil Palm with a Non-Pathogenic Malaysian Isolate of *F. oxysporum*.

It is possible that since Malaysian strains of *F. oxysporum* have been isolated from the roots (Ho *et al.*, 1985a) and stems (Flood *et al.*, 1989) of inoculated palms that they may prevent the establishment of, or cross protect palms against, Foe. Such cross protection has been reported by Renard (1976) and Taquet (1985) who found that inoculation of seedlings with non-pathogenic African isolates of *F.oxysporum* before inoculation with the pathogenic strain resulted in a significant reductions in disease. Therefore, the ability of Malaysian strains to protect against Foe was therefore tested by preinoculation of susceptible seedlings with a Malaysian isolate of *F. oxysporum*.

In these two experiments, a Malaysian isolate (LEY, from oil palm plantation soil) was used; this isolate had previously been shown to systemically colonise inoculated palms without producing disease symptoms (Flood *et al.*, 1989). Preinoculation with LEY was performed on seedlings (cross 583/5 x 583/5) at the first leaf stage by root dipping in a spore suspension (1×10^8 spores ml^{-1}) for 10 minutes before replanting, followed by soil inoculation with 10 ml of spore suspension (3.3×10^6 spores ml^{-1}). Seedlings that were not preinoculated received 10ml of 10% sucrose salts media (see Appendix 1), in order to simulate the effect of growth media on soil microflora.. After 3 weeks, all the preinoculated and half the non-

preinoculated palms were inoculated with the F3 isolate of Foe at low dose (2.2). Two hundred and twenty five days post inoculation, disease development was assessed by measurement of plant height, dry weight, browning of stem tissue and reisolation from stem tissue (2.3.1a, 2.3.1e, 2.3.2b & 2.3.3).

Inoculation with F3 alone resulted in significant reductions in plant height and dry weight (Table 61); the pathogen was isolated from all these plants and induced significant levels of stem browning (Table 62).

Table 61: Effect of Preinoculation with a Malaysian Isolate of *F. oxysporum* on Growth of Infected Palms.

	Plant Height (mm)	Dry Weight (g) Roots	Aerial Tissue
Control *	1008a	13.60a	50.48a
F3 *	799b	7.24b	30.04b
F3 + LEY *	1027a	10.87ab	49.39a

Values are the mean of 14 replicate plants, 225 days post inoculation with F3.
* Plants were either uninoculated (control), inoculated with a pathogenic isolate (F3), or preinoculated with a Malaysian isolate (LEY) before the pathogenic isolate (F3).
Within each column figures with the same letter are not significantly different ($P > 0.05$, Kruskall Wallis, and repeated Mann Whitney U-test).

However, in preinoculated plants there was no significant reduction in plant growth (Table 61) and although *F.oxysporum* was reisolated from 50% of stems there was little or no browning of stem tissue (Table 62). Thus, reduction in disease may have been associated with

colonisation with LEY, this possibility was investigated in a further experiment using UF4 clonal plants inoculated as previously described.

Table 62: Effect of Preinoculation with a Malaysian Isolate of *F. oxysporum* on Internal Symptoms and Frequency of Reisolation of *a Pathogenic F. oxysporum Isolate.*

	Browning of Stem Tissue		Reisolation Frequency
	% Area	No Plants With:Without	No Plants With : Without *F. oxysporum* $
Control *	0	0:14a	0:14a
F3 *	21.2a	11:13b	14:0c
LEY + F3 *	0.75b	4:10a	7:7b

Values are the mean of 14 replicate plants, 225 days post inoculation with F3.
* Plants were either uninoculated (control), inoculated with a pathogenic isolate (F3), or preinoculated with a Malaysian isolate (LEY) before the pathogenic isolate (F3).
$ Reisolation from stem tissue.
Within each column, figures with the same letter are not significantly different (P > 0.01, Percentage browning : Mann Whitney U-test, Frequency of brown stem tissue or reisolation : Chi-squared and Fisher's Exact test).

The degree of host colonisation by LEY was also assessed by inoculating some plants (at the time of preinoculation) with this isolate alone. Disease development was assessed (227 days post inoculation with F3) by measurements of dry weight, browning of stem tissue and reisolation from stem tissue (2.3.1e, 2.3.2b & 2.3.3).

In this experiment, preinoculation caused no reduction in either the frequency or severity of disease symptoms

(Table 63). Also, *F. oxysporum* was not isolated from any of the plants that had been inoculated with LEY alone. Therefore preinoculation may have been unsuccessful since LEY has previously been shown to colonised UF4 clonal palms to a high degree (Flood *et al.*, 1989).

Table 63: Effect of Preinoculation with a Malaysian Isolate of *F. oxysporum* on Symptom Development in Infected UF4 Clonal Palms.

	Growth of Aerial Tissue	Reisolation of *F. oxysporum* $	Stem Browning
	Dry Wt (g)	Ratio of Plants With : Without	
Control *	44.87ab	0:14a	0:14a
LEY *	49.34a	0:14a	0:14a
Ley + F3 *	33.61b	10:2b	9:3b
F3 *	33.61b	8:4b	8:4b

Values are the mean of 14 replicate plants, 227 days post inoculation with F3.
* Plants were either uninoculated (control), inoculated with a pathogenic (F3) or Malaysian (LEY) isolate, or preinoculated with LEY before F3.
$ Reisolation from stem tissue.
Within each column, figures with the same letter are not significantly different (Dry weight $P > 0.05$, Kruskall Wallis and repeated Mann Whitney U-test. Reisolation and Stem browning $P > 0.01$, Chi-squared and Fisher's Exact test).

5.3. DISCUSSION.

F. oxysporum is generally regarded as a soil-borne saprophyte (Booth, 1970). Localised spread of the fungus is achieved mostly by irrigation/flood water, water splash, water and wind erosion of soil or movement of farm equipment (Green, 1981; Nelson, 1981; Gambogi, 1983). However, dissemination over longer distances may occur by movement of infected plants, plant debris or wind blown particles. Another significant source of long distance spread is on infected or infested seeds (Gambogi, 1983).

Although *F. oxysporum* is ubiquitous in both natural and agricultural soils, the majority of isolates from these sources do not cause disease in any known host (Correll, Puhalla & Schneider, 1986; Elias, Schneider & Lear, 1991). In this and in a previous study (Renard, 1967) plantation soil isolates were also found to be non-pathogenic to oil palm. This contrasts with the results of Locke (1972) who suggested that all soil isolates were pathogenic, but his conclusion was based on reisolation of the fungus from root tissue and not on symptom development and may therefore be incorrect.

The absence (or rarity) of pathogenic isolates of *F. oxysporum* in the soil environment may indicate a low ability to compete as a soil saprophyte. However, Park (1958) demonstrated that Foe had good sapropyhtic ability in *in vitro* soil cultures and concluded that it was an effective soil inhabitant. Also, Turner (1992) in a study

of population dynamics in soil demonstrated that Foe and a soil isolate of *F. oxysporum* could be reisolated in similar quantities from the soil and rhizosphere of palms inoculated with both isolates. Therefore, Foe may be present at significant levels in plantation soils but is difficult to detect amongst the large number of non-pathogenic isolates.

In contrast, all isolates of *F.oxysporum* obtained from the seed kernels, male inflorescence, air samples, and pollen (2 from each source) were pathogenic (this study and Flood *et al.*, 1990). These observations were based on the testing of a limited number of isolates (4 from soil and 8 non-soil) but the chances of all the soil isolates being non-pathogenic whilst the non-soil isolates were pathogenic are low ($P < 0.005$, Fisher's Exact test). Additionally, Renard (pers comm, 1991) has tested a large number of soil isolates and estimated that < 1 per 1,000 were pathogenic. A further link between these non-soil isolates living as saprophytes and isolates from diseased palms was established in an investigation of vegetative compatibility groups of a range of Zairean isolates (Flood *et al.*, 1992). In their study, isolates from pollen and seed were compatible with each other and those from diseased palms, but not with soil isolates.

These results suggest that pathogenicity and the ability to inhabit certain saprophytic environments other than soil, may be related. One possible link might be through systemic colonization of inflorescences in

diseased palms, but in a limited survey (Cooper *et al.*, 1989) *F. oxysporum* was not reisolated from inside any inflorescences on diseased palms. While in this study the fungus was found at very high levels (7×10^6 propagules per inflorescence) on male inflorescences on symptomless palms. Therefore, the isolates of Foe that contaminate seed and pollen probably do not originate from diseased palms.

Hardon and Turner (1967) demonstrated that male inflorescences produced pollen for *ca.* 5 days post anthesis. However, after 3 days, there was a substantial reduction in both the quantity and viability of the pollen released, which coincided with the growth of fungal mycelium (mostly *Fusarium* spp) over the inflorescence. If this fall in pollen production was caused by the fungal colonisation this would exert a very strong selection pressure on palms to prevent or delay fungal growth, possibly through the production of antifungal chemicals. Isolates of Foe, whose pathogenicity may be due to an ability to tolerate or suppress such host defences, may therefore have a competitive advantage over other saprophytic colonisers. This possible link between pathogenicity and the ability to colonise male inflorescences could be tested by a study of the population dynamics of pathogenic and non-pathogenic isolates inoculated onto young inflorescences. These isolates could be distinguished using vegetative

incompatibility as determined by nit mutants (Flood *et al.*, 1992)

Fusarium wilt of oil palm has traditionally been regarded as a soil-borne disease in which spread is due to root contact with dead, infected palm tissue (Prendergast, 1957; Renard & Quillec, 1983; Renard & de Franqueville, 1989). However, the pathogen has been found in plantation air (Cooper *et al.*, 1989) and in this study in very large quantities on old male inflorescences. The form(s) in which Foe exists during aerial spread is (are) unknown; the pathogen may exist as free spores, mycelium or spores attached to or inside pollen, or on pollinating weevils (*Elaeidiobius* spp). Whatever the mechanism it is clear that aerial spread does occur and appears to be the mechanism by which male inflorescences become contaminated. Aerial spread of the disease would be difficult to prove in Africa, where Foe may already be present in the soil. However, with the isolated and relatively new outbreaks of wilt in S. America it may be possible to follow the spread of the pathogen through a plantation (Flood, pers comm, 1992) and to this end, samples of male inflorescence and pollen from these areas have been requested for pathogenicity testing.

Spores produced on male inflorescences may enhance the soil inoculum level around the base of palms to a level sufficient to cause infection, and yet neither of the 2 soil isolates from this area tested in this study were pathogenic.

Large scale aerial sporulation and spread is rare in *Fusarium* wilt diseases. Only in *Fusarium* wilt of Mimosa has such significant sporulation been reported; in this case from lenticels on infected trees (Phippes & Stipes, 1976). While in *Verticillium* wilt of alfalfa, although profuse sporulation of the pathogen has been observed on infected plants (Isaac, 1957), this was not thought to result in significant levels of airborne conidia (Jimenez, Diaz & Millar, 1988).

The situation with Foe is different to the above examples in that the pathogen exists as a saprophyte on the inflorescence of uninfected trees. Furthermore, the large size of the palm male inflorescence, which enables aerial pollination, also permits the production and dispersal of very large quantities of inoculum.

Vascular wilt pathogens can also be spread over larger distances on seeds, and have frequently been reported as external or internal contaminants. Seeds may be infected by systemic colonisation of the host or from external sources (Nelson, 1981; Gambogi, 1983; Sackston, 1983).

With *Fusarium* wilt of oil palm, the pathogen may have been spread to S. America on contaminated seed (see Introduction) and in this study some seeds, artificially infested with Foe, developed wilt symptoms. However, in the Ivory Coast where many thousands of seeds have been germinated, wilt symptoms have never been observed in uninoculated seedlings (Renard, pers. comm, 1991). Also

large quantities of seed have been exported from Africa to Malaysia without apparent introduction of the pathogen.

The frequency of seed transmission of vascular wilt diseases to new areas can vary and may reflect the relative conducive or suppressive nature of the soil into which the seeds are introduced (Snyder & Smith, 1981; Louvet *et al.*, 1981). Possible reasons for this lack (or rarity) of seed-borne spread may be that soil microflora prevent the establishment of Foe by competition or antagonism. However, Gambogi (1983) suggested that wilt pathogens may escape the antagonistic action of soil microflora when the pathogen is internally seed-borne (as with oil palm seeds), because host infection can occur without the need to become established in the soil environment first.

Alternatively, non-pathogenic soil isolates of *F. oxysporum* may stimulate host defence responses (Taquet, 1985). In this study, preinoculation of palm seedlings with a non-pathogenic isolate of *F.oxysporum* from Malaysian soil greatly reduced symptom development. This effect was not observed in a second experiment, but this may be explained by the failure of the non-pathogenic isolate to become established in the host.

Under more natural circumstances, the soil microflora may effectively suppress any seed-borne pathogen. Foe would also have to compete with the other microganisms that are present on seeds, and in this study *F. solani* was

always found at much higher levels (> x10) than *F. oxysporum* on naturally infested seed and pollen.

Protection of plants by preinoculation with nonpathogens has been demonstrated in a number of diseases, including wilt diseases (Matta, 1971; Matta & Garibaldi, 1983). In most preinoculation experiments with vascular wilt diseases, the pathogen is applied at the same time or only a few days after the nonpathogen (Wymore & Baker, 1982; Hillocks, 1986; Mandell & Baker, 1991; Martyn, 1991), and an increase in this interval (to 34 days) with *Fusarium* wilt of tomato gave no cross protection (Wymore & Baker, 1982). However, in the seedling experiment reported here, there was a significant reduction in infection with a 21 day interval which indicates that the isolate used had good cross protection abilities.

Matta (1971) suggested three general mechanisms for cross protection; competition for nutrients, competition for infection sites and the induction / enhancement of host defence responses. The best protection may be achieved by organisms that penetrate the host (without causing disease) and cause cross protection through a combination of all 3 mechanisms. Such protection was demonstrated with a non-pathogenic, yet invasive, strain of *F. oxysporum* against *Fusarium* wilt of cucumber (Mandeel & Baker, 1991). The isolate (LEY) used in this study has been shown to penetrate and colonise host tissue (Flood *et al.*, 1989) and therefore may have reduced disease by a

similar combination of effects. However, a more extensive study (based on vegetatively incompatible isolates) of the population dynamics of *F. oxysporum* isolates in the soil and rhizosphere and of host responses would be necessary to demonstrate this.

An alternative method of pathogen spread may be on pollen, which in this study was shown to be contaminated with Foe. The author knows of no other case where a vascular wilt pathogen has been routinely isolated from pollen, but this may be because pollen is not generally collected or produced in such quantities in other crops. However, *V. albo atrum* has been shown to infect alfalfa pollen *in vitro* (Huang & Kokko, 1985) and may be spread on pathogen contaminated pollinating insects (Huang, Hanno & Kokko, 1985).

This work has demonstrated that Foe can easily be isolated from male inflorescences, where it appears to have a competitive advantage over other non-pathogenic isolates of *F. oxysporum* and from which vast quantities of spores are released. Thus the application of contaminated pollen could introduce Foe into a niche in which it may easily become established. The risk of disseminating Foe to new areas may therefore be greater with pollen than seeds, as Foe appears to have no competitive advantage over soil microflora.

Although it was demonstrated that isolates of *F. oxysporum* from male inflorescences were pathogenic to

plantlets, infection of field palms from inoculum produced on male inflorescences has not been demonstrated. Therefore, the introduction of Foe into the air/inflorescence environment of a wilt-free area, such as Malaysia, may not necessarily introduce the disease to that area. Indeed, many kilos of pollen were imported into Malaysia from Nigeria in the 1950s (Rosenquist, 1992, pers comm), and there has been no incidence of wilt in Malaysia. The pathogenicity of isolates of *F. oxysporum* on male inflorescences in such areas where African pollen has been released could however be investigated.

However, decontamination methods are required as a precautionary measure to prevent any further spread of this disease and to facilitate the world-wide distribution of breeding material. The techniques developed in this study relied upon novel methods of fungicide application to seeds (vacuum infiltration) and pollen (in organic solvents). Captafol effectively eradicated *F. oxysporum* from artificially infested seeds without adversely affecting germination or seedling development, and the procedure of infiltration and soaking could be incorporated into standard seed germination methods. On pollen, Captafol and Daconil effectively controlled *F. oxysporum*, but their effects on pollen germination could not be assessed.

In the past, quarantine procedures for pollen have involved plating samples directly onto *Fusarium*-selective medium (Porter, pers comm, 1989). However, in this

investigation *F.solani* was always found at higher levels (often > x10) on pollen (and seed) than *F. oxysporum*, and could therefore mask the observation of *F. oxysporum*. To overcome this problem, dilution series were performed, but this procedure also diluted the fungicide applied to pollen to a level at which fungal growth was no longer suppressed. A better procedure was to directly plate material onto *Fusarium*-selective medium and presume the presence of any *Fusarium* spp indicated contamination with Foe. However, the removal of Captafol antifungal activity by dilution indicated that this fungicide did not immediately kill all fungal propagules. The lack of persistence of Captafol could even make seeds more susceptible to recolonisation by Foe by removing competitive microorganisms. Further experiments into the persistence and activity of fungicides on treated oil palm material are therefore required.

Since these preliminary experiments were performed, Captafol has been reported as a possible carcinogen and has been withdrawn (Anon, 1990). However, for seed treatment, vacuum infiltration with Sportak alpha (Prochloraz & Carbendazin [1g a.i. l^{-1}]) has recently produced promising results in this laboratory (Turner, 1992), as has pollen treatment with Cyclohexane plus Sportak alpha (1g a.i. l^{-1}) (Adams, 1991; Stathers, 1992). Further experiments into these 2 treatments and the use of Daconil are required.

Organic solvents were used in this investigation as a method of applying fungicides to pollen without causing rehydration. However such solvents can frequently carry a much higher concentration of dissolved fungicide than water (saturated solution of Benomyl in water 2 mg l^{-1}, in ethanol 4 g l^{-1} [Worthing & Walker, 1983]) and may stimulate chlamydospore germination (Griffin, 1981). Therefore, the incorporation of solvents into seed fungicide treatments may also be worthwhile.

In summary, little is known of the epidemiology of this disease. However, this study highlights the potential for rapid aerial spread and multiplication on male inflorescences, which may have considerable implications in plantations which could become increasingly based on genetically uniform clonal material. Also, there is a clear potential for spread to new areas on seed and pollen, but techniques for decontamination should be available from this laboratory in the near future. Furthermore, the recent development of nit mutant techniques to differentiate between vegetative compatibility groups may alow rapid differentiation between pathogenic and nonpathogenic isolates, without the need for prolonged pathogenicity trials, ~~will~~ and could. facilitate the large scale screening of *F. oxysporum* isolates from plantations and from seed and pollen.

6. GENERAL DISCUSSION

Most investigations of vascular wilt diseases have employed artificial inoculation of susceptible material; this has led to the rapid development of severe symptoms, which have frequently been attributed to water stress. However, under natural conditions, infection and subsequent symptom development may be less acute. *Fusarium* wilt of oil palm is unusual in that even with heavily inoculated plants symptom development is very slow. Therefore, with this disease it was possible to perform an in depth study of the physiology of chronically diseased plants, which may also be relevant to the situation with naturally infected plants with some other vascular diseases.

Severe leaf stunting that is frequently observed in diseased palms has previously been attributed to host water stress. However, this study has demonstrated that while such stunted leaves were suffering from prolonged mild water stress, there was no reduction in the rate of photosynthesis or transpiration per unit leaf area. In contrast, leaves formed before the pathogen becomes established, and therefore not stunted, suffer from very severe, permanent, water stress. Calculated values of xylem resistivity in the petiole of these leaves were very high, yet very few xylem vessels were colonised by the pathogen, or showed any signs of occlusion. In the petioles of stunted leaves there were also few signs of

xylem colonisation, but xylem vessels were much reduced in diameter. This would be expected to greatly increase vascular resistance, yet measurements indicate that this was largely off set by the reduction in leaf size.

These results might suggest that the high vascular resistivity in the petioles of non-stunted leaves was due to embolisms, to which the narrower vessels in stunted leaves are more tolerant. However, although stunting has been correlated to reduced water stress, there is no experimental evidence for embolisms occurring in diseased palms, or for the resistance of narrow vessels to embolism. Both these theories require further investigation.

Stunting was primarily due to a reduction in cell division, and it could be simulated by the application of an inhibitor of gibberellin synthesis to uninfected plants. Conversely, stunting was partially redressed by applying GA3 to infected plants. Therefore, by reducing leaf area and increasing resistance to embolisms, stunted leaves may represent an adaptation to disease-induced water stress caused by a reduction in host gibberellin. If narrow vessels are an adaptation to host water stress they may also occur in other wilt diseases, and have been observed in *Verticillium* wilt of alfalfa (Pennypacker & Leath, 1986), but the author knows of no other similar reports. This possibility should be investigated, particularly in diseases where symptoms develop

comparatively slowly, for example, *Verticillium* wilt in inoculated cocoa seedlings.

Limited observation of the stomatal conductance and photosynthesis of infected field palms suggested that they react in a manner similar to infected seedlings and plantlets. However, ideally the water relations of field palms need to be measured more directly than was possible in this investigation. Also, the effect of infection on xylem vessel diameter in field palms needs investigation, especially in comparing the acute, chronic and latent forms of disease. These observations may explain why some field palms die quickly, with no growth adaptation, while others take years to die.

Although these experiments have provided circumstantial evidence for reductions in host GA levels as the cause of stunting, this theory can only be fully supported by direct measurement of plant GA levels. If a GA synthesis inhibitor is produced by the pathogen it may be obtainable from culture filtrates, and the effect of this chemical(s) on the growth and water stress tolerance of oil palm and other plant species could be tested. If, as in diseased palms, the putative GA inhibitor reduced xylem diameter then (although this would increase vascular resistance) it could be regarded as a desirable characteristic for the avoidance of drought induced embolism. Indeed, the production of drought resistant cereals with narrow xylem vessels is being attempted (Richards & Passioura, 1989). However if, as seems probable, reduced xylem diameter was

accompanied by a decreased leaf area then crop yield may be impaired. Although, under conditions of severe drought, some crops (*e.g.* maize) with a high water requirement may die and for such plants reductions in xylem diameter and leaf area may be worthwhile. The usefulness of PBZ to reduce crop water loss may be constrained by the cost of the chemical and its application. However, if the gene(s) for the production of the putative PBZ-like chemical by Foe could be identified, it may be cloned into plant species to produce more drought tolerant crops.

Although observations of the physiological mechanisms of symptom development may increase our general understanding of this and other vascular diseases, they are unlikely to contribute significantly towards a reduction of *Fusarium* wilt of oil palm in Africa. The most obvious strategy for disease control remains the breeding of palms for disease resistance.

This study has improved upon existing nursery testing procedures, and developed what is now a dependable method for the routine screening of clonal material. However, although such improvements are essential, the ability to routinely produce infected plants in the glasshouse is equally important as a technique for increasing our knowledge of the mechanisms and inheritance of disease resistance, and of variation in the pathogenicity and virulence of Foe isolates. For example, following reports of resistant material from one area being susceptible to Foe in other countries (Oritsejafor, 1989; Aberungboye,

1981; Renard, 1991, pers comm) the possibility of races of Foe is being examined here by inoculation of 16 clones with isolates from 3 different parts of Africa. Also, the segregation of resistance genes is being investigated by inoculation of full, half and three quarter crosses of a possibly immune Dumpy palm. By RFLP analysis (to be performed by S. Mayes at Plant Breeding International, Cambridge) of resistant and susceptible palms of these crosses it may be possible to identify areas of DNA associated with disease resistance.

This study has also increased the reliability of the field trial through the development of a new increment borer technique for sampling from the trunks of mature palms. This technique revealed that latent infection was common in palms of some crosses while others were totally uninfected. Unfortunately, this survey was limited, and thus the frequency of such infection, both on a plantation scale and within different crosses or clones, plus its effect on yield requires further investigation. This technique could also be used to investigate if the low incidence of wilt reported in semi-wild groves is due to a low level of Foe or a high level of latent infection.

Although this study has improved the reliability of resistance screening at the nursery and field stage, these experiments still take several months (nursery) or years (field) to perform. Furthermore, the absence of infection in a palm may indicate merely that it was not sufficiently

challenged, while infection of an otherwise resistant plant may result from an exceptionally severe challenge. Therefore, a rapid model system is required in which all plants are inoculated uniformly. Such a system, involving infiltration under reduced pressure of Foe spores into petiole sections, has recently been developed by the author and others in this laboratory, and has clearly distinguished between 3 resistant and 3 susceptible clones in only 8 days. This method has obvious advantages for the rapid screening of crosses and clones but more importantly, it will now be possible to test the resistance of individual high-yielding palms in the field, and to investigate the segregation of resistance within crosses. This would substantially increase our understanding of the inheritance of resistance.

Using this technique it may also be possible to rapidly distinguish between pathogenic and non-pathogenic isolates of *F. oxysporum*. At the moment this can only be done by pathology trials or by studying the vegetative compatibility of nitrate non-utilising mutants (Flood *et al.*, 1992). Such a rapid test would have been very useful to the epidemiological experiments performed in this study. These experiments demonstrated that although soil isolates of *F. oxysporum* were non-pathogenic, those from the air, pollen samples and old male inflorescences were pathogenic. These results have important implications for the spread of what had previously been regarded as a soil or seed-borne disease.

The prevalence of Foe on pollen and male inflorescences might suggest that pollen contains antifungal substances to which Foe was more tolerant than non-pathogenic forms of *F. oxysporum*. This theory could be tested by studying the effects of pollen extracts on the growth of Foe and on non-pathogenic isolates; if the correlation was clear, then tolerance to pollen extracts could provide an alternative, and very rapid, pathogenicity test. Such a test would permit a more extensive epidemiological study of this disease, and may be used to demonstrate whether the low incidence of wilt in semi-wild groves is correlated with a low level of Foe in that environment.

Fusarium wilt of oil palm has almost certainly been spread to S. America on contaminated seed (Flood *et al.*, 1992)), and although it may be possible to distinguish between pathogenic and non-pathogenic isolates of *F. oxysporum*, for quarantine purposes, all isolates of this fungus should be eradicated from seed and pollen samples. Consequently, two novel systems for the decontamination of both seed and pollen samples were developed, which should facilitate the export from Africa of guaranteed pathogen-free seed and pollen. One of the techniques, vacuum infiltration of seeds, may also be used to infiltrate plant growth substances into the region of the embryo, where they could break seed dormancy. Treatment with plant growth substances has been used successfully to break seed dormancy in several plant species (Bradbeer, 1988). If this procedure, which is currently under investigation,

were successful it would circumvent the present practice of heat treatment of seeds for 60 days to break seed dormancy.

In conclusion, oil palm is an important food crop in W. Africa, where a significant proportion of the oil is harvested from low yielding semi-wild palms. In part, the productivity of palms in these areas, and on commercial plantations, could be improved by the introduction of higher yielding crosses or clones, but such material must first be screened for resistance to *Fusarium* wilt. In several African countries, programmes for developing and distributing such high yielding resistant material are in operation (Hartley, 1988; Porter, 1989). In addition, in South America, where the disease is likely to become more important, screening for disease resistance is essential. However, in part such programmes have been constrained by a lack of understanding of the genetics of the host-pathogen interaction and the slow and variable development of symptoms in trials for the determination of disease resistance. The developments described in this thesis along with the other recent model system in this laboratory, are likely to make a significant contribution to the development and eventual understanding of resistance of oil palm to *Fusarium* wilt.

7. References

Adams R. 1991. *Investigation of Fusarium Wilt of Oil Palm.* Report for Honours Degree in Applied Biology. University of Bath.

Aderungboye FO. 1981. Significance of vascular wilt in oil palm plantations in Nigeria. In: Pushparajah E, Chew Pol Sew, eds. *The Oil Palm in Agriculture in the Eighties*. Kuala Lumpar: Incorporate Society of Planters, 1-11.

Agrios GN. 1988. *Plant Pathology*. Third edition. New York: Academic Press.

Aloni B, Pashkar T. 1987. Antagonistic effects of paclobutrazol and gibberellic acid on growth and some biochemical characteristics of pepper (*Capsicum annuum*). *Scientia Horticulturae* **33**: 167-177.

Anon. 1951. Jaarverslag over 1950. *Department of Agriculture. Surinam.* 1950 p87. In: *Review of Applied Mycology* **32**: 546-547. (abstract).

Anon. 1960. *Annual Report of the Cameroon Development Corporation.* 1959. p29. In: *Review of Applied Mycology* **40**: 148. (abstract).

Anon. 1990. Ivens GW, ed. *The UK Pesticide Guide*. Wallingford: Commonwealth Agricultural Bureaux, and Farnham: British Crop Protection Council.

Anon. 1991. Oil palm development in Nigeria: status and prospects. In: Anon, 1991. The Role of Research Networks for the Development of Oil Palm in Africa. AFOPDA/BUROTROP seminar. Abidjan. June 1991. Paris. BUROTROP.

Bachy A. 1970. La fusariose du palmier a huile. *Oleagineux* **25**: 265-267.

Baker DA. 1984. Water Relations. In: Wilkins MB, ed. *Advanced Plant Physiology*. London: Pitman Publishing.

Ballio A. 1978. Fusicoccin the vivotoxin of *Fusicoccum amygdali*. Chemical properties and biological activity. *Annales de Phytopathologie* **10**, 145-156.

Barrett JN, Potulski N, Smith BG. 1987. *Drought Effects on Oil Palm Ramets, Part 3*. Unilever Research Colworth Laboratory, **Report XCW 87 0257.**

Barrett JN, Smith BG, Jones LH. 1985. *Drought Effects on Oil Palm Ramets, Part 1*. Unilever Research Colworth Laboratory, **Report PCW 85 1236.**

Beckman CH. 1964. Host responses to vascular infection. *Annual Review of Phytopathology* **2**: 231-252.

Beckman CH. 1964. Host responses to vascular infection. *Annual Review of Phytopathology*. **2**: 231-252.

Beckman CH. 1987. *The Nature of Wilt Diseases of Plants*. St Paul: American Phytopathological Society.

Beckman CH, Halmos S, Mace ME. 1962. The interaction of host, pathogen and temperature in relation to susceptibility to *Fusarium* wilt in banana. *Phytopathology* **52**: 134-140.

Beckman CH, Kuntz JE, Riker AJ, Berbee JG. 1953. Host responses associated with the development of oak wilt. *Phytopathology* **43**: 448-454.

Beckman CH, Verdier PA, Mueller WC. 1989. A system of defence in depth provided by vascular parenchyma cells of tomato in response to vascular infection with *Fusarium oxysporum* f.sp. *lycopersici*, race 1. *Physiological and Molecular Plant Pathology* **34**: 227-239.

Bishop CD, Cooper RM. 1983. An ultrastructural study of vascular colonisation in three vascular wilt diseases I. Colonisation of susceptible cultivars. *Physiological and Plant Pathology* **23**: 323-343.

Black CR. 1979. The relative magnitude of the partial resistances to transpirational water movement in sunflower (*Helianthus annus* L). *Journal of Experimental Botany*. **30**: 245-253.

Booth C. 1971. *The Genus Fusarium*. Kew: Commonwealth Mycological Institute.

Booth C. 1971. *The Genus Fusarium*. Kew: Commonwealth Mycological Institute.

Bowden RL, Rouse DI. 1991a. Effects of *Verticillium dahliae* on gas exchange of potato. *Phytopathology* **81**: 293-300.

Bowden RL, Rouse DJ. 1991b. Chronology of gas exchange effects and growth effects of infection by *Verticillium dahliae* in potato. *Phytopathology* **81**: 301-310.

Boyer JS. 1967. Leaf water potential measurement with a pressure chamber. *Plant Physiology*. **42**: 133-137.

Boyer JS. 1973. Response of metabolism to low water potentials in plants. *Phytopathology* **63**: 466-472.

Boyer JS. 1985. Water transport. *Annual Review of Plant Physiology* **36**: 473-516.

Boyer JS. 1989. Water potential and plant metabolism: comments on Dr P. J. Kramer's article, Changing concepts regarding plant water relations, volume 11, number 7, pp. 565-568, and Dr J. B. Passioura's response, pp. 569-571. *Plant, Cell and Environment* **12**: 213-216.

Bradbeer JW. 1988. *Seed Dormancy and Germination*. Glasgow: Blakie ans Son.

Bradford KJ, Hsiao TC. 1982. Physiological responses to moderate water stress. In: Laige OL, Nobel PS, Osmond CB, Ziegler H. eds. *Physiological Plant Ecology, vol 2* in *Encyclopaedia of Plant Physiology, vol 12b*. Berlin: Springer-Verlag, 263-313.

Buddenhagen IW. 1990. Banana breeding and *Fusarium* wilt. In: Ploetz RC, ed. *Fusarium Wilt of Banana*. St Paul: American Phytopathological Society, 107-114.

Burden RS, Carter GA, Clark T, Cooke DT, Croker SJ, Deas AHB, Hedden P, James CS, Lenton JR. 1987. Comparative activity of the enantiomers of triadimenol and paclobutrazol as inhibitors of fungal growth and plant sterol and gibberellin biosynthesis. *Pesticide Science* **21**: 253-267.

Casper JA, Taylor BH. 1989. Growth and development of young loring peach trees after foliar sprays of paclobutrazol and GA3. *Hortscience* **24**: 240-242.

Cohen R, Yarden O, Katan J, Riov J, Lisker N. 1987. Paclobutrazol and other plant growth-retarding chemicals increase resistance of melon seedlings to fusarium wilt. *Plant Pathology* **36**: 558-564.

Colhoun J. 1981. Vascular wilt disease of oil palm. In: Nelson PE, Toussoun TA, Cook RJ, eds. *Fusarium Disease, Biology and Taxonomy*. University Park: Pennsylvania State University Press, 22-28.

Cooper RM, Flood J, Mepsted R. 1989. *Fusarium* wilt of oil palm: transmission, isolate variation, resistance. In: Tjamos EC, Beckman CH, eds. *The Interaction of Genetic and Environmental Factors in the Development of Vascular Wilt Diseases of Plants*. Berlin: Springer-Verlag.

Cooper RM, Wood RKS. 1975. Regulation of synthesis of cell wall degrading enzymes by *Verticillium albo-atrum* and *Fusarium oxysporum* f.sp. *lycopersici*. *Physiological Plant Pathology* **5**: 135-156.

Cooper RM, Wood RKS. 1980. Cell wall degrading enzymes of vascular wilt fungi. III. Possible involvement of endo-pectin lyase in *Verticillium* wilt of tomato. *Physiological Plant Pathology* **16**: 285-300.

Corden MA, Chambers HL. 1966. Vascular dysfunction in *Fusarium* wilt of tomato. *American Journal of Botany.* **53:** 284-287.

Corley RHV. 1976. Photosynthesis and productivity. In: Corley RHV, Hardon JJ, Wood BJ, eds. *Developments in Crop Science (1), Oil Palm Research.* Amsterdam: Elsevier Scientific Publishing Company, 55-86.

Correll JC, Puhalla JE, Schneider RW. 1986. Vegetative compatibility groups among nonpathogenic root-colonizing strains of *Fusarium oxysporum. Canadian Journal of Botany* **64:** 2358-2361.

Cosgrove DJ, Cleland RE. 1983. Solutes in the free space of growing stem tissues. *Plant Physiology* **72:** 326-331.

Creber GT, Chaloner WG. 1984. Influence of environmental factors on the wood structure of living and fossil trees. *Botanical Review* **50,** 357-436.

Dale JE. 1988. The control of leaf expansion. *Annual Review of Plant Physiology and Plant Molecular Biology* **39,** 267-295.

Darlington AB, Dixon MA. 1991. The hydraulic architecture of roses (*Rosa hybrida*). *Canadian Journal of Botany* **69:** 702-710.

Davies WJ, Zhang J. 1991. Root signals and the regulation of growth and development of plants in drying soil. *Annual Review of Plant Physiology and Plant Molecular Biology* **42:** 55-76.

Day PR. 1974. *Genetics of Host-Pathogen Interaction.* San Francisco: Freeman and Company.

Dey R, Van Alfen N. 1979. Influence of *Corynebacterium insidiosum* on water relations of alfalfa. *Phytopathology* **69:** 942-946.

Dimond AE. 1966. Pressure and flow relations in vascular bundles of the tomato plant. *Plant Physiology* **41:** 119-131.

Dimond AE. 1970. Biophysics and biochemistry of the vascular wilt syndrome. *Annual Review of Phytopathology.* **8:** 301-322.

Dimond AE. 1972. The origin of symptoms of vascular wilt diseases. In: Wood RKS, Ballio A, Graniti A, eds. *NATO ASI Series; Phytotoxins in Plant Diseases.* London: Academic Press, 289-309.

Dimmond AE, Waggoner PE. 1953. The water economy of *Fusarium* wilted tomato plants. *Phytopathology* **43:** 619-623.

Dossa C, Pando-Bahuon A, Renard JL, Boisson C. 1991. Determination of vegetative compatibility groups in African *Fusarium oxysporum* strains isolated from vascular wilt-infected oil palms. *Oleagineaux* **46**: 145-147.

Douglas SM, MacHardy WE. 1981. The relationship between vascular alterations and symptom development in *Verticillium* wilt of chrysanthemum. *Physiological Plant Pathology*. **19**: 31-39.

Downton WJS, Loveys BR, Grant WJR. 1988. Stomatal closure fully accounts for the inhibition of photosynthesis by abscisis acid. *New Phytologist* **108**, 263-266.

Dufrene E, Ochs R, Saugier B. 1990. Oil palm photosynthesis and productivity linked to climatic factors. *Oleagineux* **45**: 345-355.

Duniway JM, Slatyer RO. 1971. Gas exchange studies on the transpiration and photosynthesis of tomato leaves affected by *Fusarium oxysporum* f.sp. *lycopersici*. *Phytopathology* **61**: 1377-1381.

Duniway JM. 1971a. Resistance to water movement in tomato plants infected with *Fusarium*. *Nature* **230**, 252-253.

Duniway JM. 1971b. Water relations of *Fusarium* wilt in tomato. *Physiological Plant Pathology* **1**: 537-546.

Elias KS, Schneider RW, Lear MM. 1991. Analysis of vegetative compatibility groups in nonpathogenic populations of *Fusarium oxysporum* isolated from symptomless tomato roots. *Canadian Journal of Botany* **69**: 2089-2094.

Evans SM, Wilson IM. 1971. The anther smut of sea campion. A study of the role of growth regulators in the dwarfing symptom. *Annals of Botany* **35**: 543-553.

Flood J, Cooper RM, Lees PE. 1989. An investigation of pathogenicity of four isolates of *Fusarium oxysporum* from South America, Africa and Malaysia to clonal oil palm. *Journal of Phytopathology* **124**: 80-88.

Flood J, Mepsted R, Cooper RM. 1990. Contamination of oil palm pollen and seed by *Fusarium* species. *Mycological Research*. **94**: 708-709.

Flood J, Mepsted R. 1988. *Fusarium Wilt of Oil Palm*. Unilever Internal Report.

Flood J, Whitehead DS, Cooper RM. 1992. Vegetative compatibility and DNA polymorphism in *Fusarium oxysporum* f.sp. *elaeidis* and their relationship to isolate virulence and origin. *Physiological and Molecular Plant Pathology*, (In Press).

Franqueville (de) H. 1984. Vascular wilt of the oil palm: relationship between nursery and field resistance. *Oleagineux* **39**: 513-518.

Franqueville (de) H. 1991. Former savannah or former forest: effect of pathogen isolate on the performance of oil palm families with respect to *Fusarium* wilt. *Oleagineux* **46**: 179-186.

Franqueville (de) H, de Greef W. 1987. Hereditary transmission of resistance to vascular wilt of the oil palm: Facts and hypotheses. *International Oil Palm Conference*. Kuala Lumpur.

Franqueville (de) H, Diabate S, Renard JL, Jacquemard JCh. 1991. Assessment of oil palm clone performance with regard to vascular wilt. *British Society for Plant Pathology, Annual Conference 1991*.

Franqueville (de) H, Renard JL. 1990. Improvement of palm vascular wilt tolerance. Results and development of the disease at the R. Michaux plantation. *Oleagineux* **45**: 399-405.

Fraselle JV. 1951. *Fusarium oxysporum* Schl. as the cause of vascular wilt disease of the oil palm (*Elaeis guineensis*). *Transactions of the British Mycological Society* **34**: 492-496.

Freemann S, Katan J. 1988. Weakening effect on propagules of *Fusarium* by sublethal heating. *Phytopathology*. **87**: 1656-1661.

Gambogi P. 1983. Seed transmission of *Fusarium oxysporum:* epidemiology and control. *Seed Science and Technology* **11**: 815-827.

Gaumann E. 1957. Fusaric acid as a wilt toxin. *Phytopathology* **47**: 342-357.

Gaumann E. 1958. The mechanisms of fusaric acid injury. *Phytopathology* **48**: 670-686.

Gerdemann JW, Finley AM. 1951. The pathogenicity of races 1 and 2 of *Fusarium oxysporum* f.sp. *lycopersici*. *Phytopathology* **41**: 238-244.

Gibson AC, Calkin HW, Nobel PS. 1984. Xylem anatomy, water flow and hydraulic conductance in the fern *Cyrtomium falcatum*. *American Journal of Botany* **71**, 564-574.

Giordano R, Salleo A, Salleo S, Wanderlingh F. 1978. Flow in the xylem vessels and Poiseuille's law. *Canadian Journal of Botany* **56**, 334-338.

Goodwin PH, DeVay JE, Meredith CP. 1988a. roles of water stress and phytotoxins in the development of Pierce's disease of grapevine. *Physiological and Molecular Plant Pathology*. **32**: 1-15.

Goodwin PH, DeVay JE, Meredith CP. 1988b. Physiological responses of *Vitis vinifera* cv. Chardonnay to infection by Pierces's disease bacterium. *Physiological and Molecular Plant Pathology*. **32**: 17-32.

Goth RW, Webb RE. 1981. Sources and genetics of resistance in vegetable crops. In: Mace ME, Bell AA, Beckman CH, eds. *Fungal Wilt Diseases of Plants*. New York: Academic Press, 377-412.

Gottleib D. 1944. The mechanism of wilting caused by *Fusarium bulbigenum* var *lycopersici*. *Phytopathology* **34**: 41-59.

Green RJ. 1981. An overview. In: Mace ME, Bell AA, Beckman CH, eds. *Fungal Wilt Diseases of Plants*. New York: Academic Press, 1-24.

Greenberger A, Yogev A, Katan J. 1987. Induced suppressiveness in solarised soils. *Phytopathology* **77**: 1663-1669.

Grier CC, Running SW. 1977. Leaf area of mature northwestern coniferous forests: relation to site water balance. *Ecology* **58**: 893-899.

Griffin GJ. 1981. Physiology of conidium and chlamydospore germination in *Fusarium*. In: Nelson PE, Toussoun TA, Cook RJ, eds. *Fusarium Disease, Biology and Taxonomy*. University Park: Pennsylvania State University Press, 331-339.

Guldentops RE. 1962. Contribution a l'etude de la tracheomycose du palmier a huile. *Parasitica* **18**: 244-263.

Hall R, Busch LV. 1971. *Verticillium* wilt of chrysanthemum: colonization of leaves in relation to symptom development. *Canadian Journal of Botany* **49**: 181-185.

Hall R, MacHardy WE. 1981. Water Relations. In: Mace ME, Bell AA, Beckman CH, eds. *Fungal Wilt Diseases of Plants*. New York: Academic Press, 255-299.

Hampton RE, Wullschleger SD, Oosterhuis DM. 1990. Impact of *Verticillium* wilt on the net photosynthesis, respiration and photorespiration in field-grown cotton (*Gossypium hirsutum* L.). *Physiological and Molecular Plant Pathology* **37**, 271-280.

Hardon JJ. 1970. Inbreeding in populations of the oil palm (*Elaeis guineensis* Jacq.) and its effect on selection. *Oleagineux* **25**: 449-456.

Hardon JJ, Turner PD. 1967. Observations on the natural pollination in commercial plantings of oil palm (*Elaeis guineensis*) in Malaya. *Experimental Agriculture* **3**: 105-116.

Hardon JJ, Williams CN, Watson I. 1969. Leaf area and yield in the oil palm in Malaya. *Experimental Agriculture* **5**: 25-32.

Harrison JAC, Isaac I. 1968. Leaf area development in king edward potato plants inoculated with *Verticillium albo-atrum* and *V. dahliae*. *Annals of Applied Biology* **61**, 217-230.

Harrison JAC. 1970. Water deficit in potato plants infected with *Verticillium albo atrum*. *Annals of Applied Biology*. **66**: 225-231.

Harrison JAC. 1971. Transpiration in potato plants infected with *Verticillium* spp. *Annals of Applied Biology*. **68**: 159-168.

Hartley CWS. 1988. *The Oil Palm*. Third edition. London: Longmans.

Hartung W, Rabin JW, Hendrix DL. 1988. Abscisic acid movement into apoplast solutions of water-stressed leaves. *Plant Physiology* **86**: 908-913.

HavErkort AJ, Rouse DI, Turkensteen LJ. 1990. The influence of *Verticillium dahliae* and drought on potato crop growth. 1. Effects of gas exchange and stomatal behaviour of individual leaves and crop canopies. *Netherlands Journal of Plant Pathology* **96**, 273-289.

Henson IE. 1991. Age-related changes in stomatal and photosynthetic characteristics of leaves of oil palm (*Elaeis guineensis* Jacq.) *Elaeis* **3**: 336-348.

Hillocks RJ. 1986. Cross protection between strains of *Fusarium oxysporum* f. sp. *vasinfectum* and its effect on vascular resistance mechanisms. *Journal of Phytopathology* **117**: 216-225.

HO YW, Varghese G, Taylor GS. 1985a. Pathogenicity of Malaysian and African *Fusarium oxysporum* isolates on Malaysian and African oil palm seedlings. *Phytopathologische Zeitschrift* **114**: 193-202.

HO YW, Varghese G, Taylor GS. 1985b. Histopathology of oil palm seedlings infected by pathogenic *Fusarium oxysporum* isolates from Africa. *Phytopathologische Zeitschrift* **114**: 312-324.

Hsiao TC. 1973. Plant responses to water stress. *Annual Review of Plant Physiology* **24**: 519-570.

Huang HC, Hanna MR, Kokko EG. 1985. Mechanisms of seed contamination by *Verticillium albo-atrum* in alfalfa. *Phytopathology* **75**: 482-488.

Huang HC, Kokko EG. 1985. Infection of alfalfa pollen by *Verticillium albo-atrum*. *Phytopathology* **75**: 859-865.

Husken D, Streudle E, Zimmermann U. 1978. Pressure probe technique for measuring water relations of cells in higher plants. *Plant Physiology*: **61**: 158-163.

Idso SB, Allen SG, Kimball BA, Choudhury BJ. 1989. Problems with porometry: Measuring net photosynthesis by leaf chamber techniques. *Agronomy Journal* **81**, 475-479.

Jain A, Shivanna KR. 1988. Storage of pollen grains in organic solvents: Effect of organic solvents on leaching of phospholoipids and its relationship to pollen viability. *Annals of Botany* **61**: 325-330.

Jones HJ. 1985. Movement of liquid water and the effects of fungal infection. In: Ayers PG, Boddy L, eds. *Water Fungi and Plants*. Cambridge: Cambridge University Press.

Jones LH. 1983. The oil palm and its clonal propagation by tissue culture. *Biologist* **30**: 181.

Kendall SJ. 1985. *Sensitivity In Vitro of Certain Fungal Pathogens of Oil Palm to Selected Fungicides*. Long Ashton Internal Report.

Koide R. 1985a. The nature and location of variable hydraulic resistance in *Helianthus annuus* L. (sunflower). *Journal of Experimental Botany 36*: 1430-1440.

Koide R. 1985b. The effect of VA mycorrhizal infection and phosphorus status on sunflower hydraulic and stomatal properties. *Journal of Experimental Botany*. **36**: 1087-1098.

Kovachich WG. 1948. A preliminary anatomical note on vascular wilt disease of the oil palm (*Elaeis guineensis*). *Annals of Botany* **12**: 327-330.

Kramer PJ. 1988. Changing concepts regarding plant water relations. *Plant, Cell and Environment* **11**, 565-568.

Kullaya A. 1991. Oil palm production in Tanzania. In: Anon, 1991. The Role of Research Networks for the Development of Oil Palm in Africa. AFOPDA/BUROTROP seminar. Abidjan. June 1991. Paris. BUROTROP.

Kuroda K. 1991. Mechanism of cavitation development in the pine wilt disease. *European Journal of Forest Pathology* **21**, 82-89.

Ladiges PY. 1975. Some aspects of tissue water relations in three populations of *Eucalyptus viminalis* Labill. *New Phytologist* **75**: 53-62.

Lande (van de) HL. 1984. Vascular wilt disease of oil palm (*Elaeis guineensis*) in Para, Brazil. *Oil Palm News* **28**: 6-10.

Larson PR. 1963. The indirect effects of drought on tracheid diameter in red pine. *Forest Science* **9**, 52-62.

Lichtenthaler HK, Wellburn AR. 1983. The extraction and quantification of photosynthetic pigments. *Biochemical Society Transactions* **11**, 591-592.

Lo Gullo MA, Salleo S. 1991. Three different methods for measuring xylem cavitation and embolism: A comparison. *Annals of Botany* **67**: 417-424.

Locke T. 1972. *A Study of Vascular Wilt Disease of Oil Palm Seedlings*. PhD Thesis, University of Manchester.

Locke T, Colhoun J. 1973. *Fusarium oxysporun* f.sp. *elaeidis* as a seed-bourne pathogen. *Transactions of the British Mycological Society*. **60**: 594-595.

Locke T, Colhoun J. 1974. Contributions to a method of testing oil palm seedlings for resistance to *Fusarium oxysporum* Schl. f.sp. Toovey. *Phytopathologische Zeitschrift* **79**: 77-92.

Locke T, Colhoun J. 1977. A process of infection of oil palm seedlings by *Fusarium oxysporum* f.sp. *elaeidis*. *Phytopathologische Zeitschrift* **88**: 18-28.

Louvet J. 1988. *Fusarium oxysporum*. In: Smith IM, Dunez J, Phillip DH, Lelliot RA, Archer SA, eds. *The European Handbook of Plant Diseases*. London: Blackwell Scientific Publications, 289-292.

Louvet J, Alabouvette C, Rouxel F. 1981. Microbiological suppressiveness of some soils to *Fusarium* wilts. In: Nelson PE, Toussoun TA, Cook RJ, eds. *Fusarium Disease, Biology and Taxonomy*. University Park: Pennsylvania State University Press, 261-275.

MacHardy WE, Busch LV, Hall R. 1976. *Verticillium* wilt of chrysanthemum: quantitative relationship between increased stomatal resistance and local vascular dysfunction preceding wilt. *Canadian Journal of Botany* **54**: 1023-1034.

MacHardy WE, Hall R, Busch LV. 1974. *Verticillium* wilt of chrysanthemum: relative water content and protein, RNA and chlorophyll levels in leaves in relation to visible wilt symptoms. *Canadian Journal of Botany* **52**: 49-54.

Mandeel Q, Baker R. 1991. Mechanisms involved in biological control of Fusarium wilt of cucumber with strains of nonpathogenic *Fusarium oxysporum*. *Phytopathology* **81**: 462-469.

Martyn RD. 1991. Induced resistance to Fusarium wilt of watermelon under simulated field conditions. *Plant Disease* **75**: 874-877.

Mathre DE. 1968. Photosynthetic activities of cotton plants infected with *Verticillium albo atrum*. *Phytopathology* **58**: 137-141.

Matta A. 1971. Microbial penetration and immunization of uncongenial host plants. *Annual Review of Phytopathology* **9**: 387-410.

Matta A, Garibaldi A. 1977. Control of Verticillium wilt of tomato by preinoculation with avirulent fungi with avirulent. *Netherlands Journal of Plant Pathology* **83**: 457-462.

Mead R, Curnow RN. 1983. *Statistical Methods in Agriculture and Experimental Biology*. London: Chapman and Hall.

Meinzer FC, Moore PH. 1988. Effect of apoplast solutes on water potential in elongating sugarcane leaves. *Plant Physiology* **86**: 873-879.

Mepsted R, Nyanduza C, Flood J, Cooper RM. 1991. A non-destructive quantitative method for the assessment of infection of oil palms by *Fusarium oxysporum* f.sp. *elaeidis*. *Elaeis* **3**: 329-335.

Meunier J, Renard JL, Quillec G. 1979. Heredity of resistance to *Fusarium* wilt in the oil palm *Elaeis guineensis* Jacq. *Oleagineux* **34**: 555-561.

Milburn JA. 1973a. Cavitation in *Ricinus* by acoustic detection: induction in excised leaves by various factors. *Planta* **110**: 253-265.

Milburn JA. 1973b. Cavitation studies on whole *Ricinus* plants by acoustic detection. *Planta* **112**: 333-342.

Misaghi IJ, DeVay JE, Duniway JM. 1978. Relationship between occlusion of xylem elements and disease symptoms in leaves of cotton plants infected with *Verticillium dahliae*. *Canadian Journal of Botany* **56**: 339-342.

Mishio M, Yokoi Y. 1991. A model for estimation of water flow resistance in soil-leaf pathway under dynamic conditions. *Journal of Experimental Botany 42*: 541-546.

Mishra R, Shivanna KR. 1985. Storage of pollen in organic solvents-an assessment. In: Varghese TM, ed. *Recent Advances in Pollen Research.* New Delhi: Allied Publishers Private.

Mollenhauer HH, Hopkins DL. 1976. Xylem morphology of Pierce's disease-infected grapevines with different levels of tolerance. *Physiological Plant Pathology* **9**: 95-100.

Morgan JM. 1984. Osmoregulation and water stress in higher plants. *Annual Review of Plant Physiology* **35**, 299-319.

Mouya I. 1992. Caracterisation de plusieurs *Fusarium oxysporum* pathogenes de trois especes de palmiers par la techniques RFLP. In Renard JL, ed. *Mecanismes des Reacions de Defense du Palmier a Huile vis-a-vis du Fusarium oxysporum f.sp. elaeidis,* Doc no 2415. Montpellier: Institut de Recherches pour les Huiles et Oleagineux. PhD Thesis, University Pierre et Marie Curie, Paris.

Nelson PE. 1981. Life cycle and epidemiology of *Fusarium oxysporum*. In: Mace ME, Bell AA, Beckman CH, eds. *Fungal Wilt Diseases of Plants*. New York: Academic Press, 51-80.

Newbanks D, Bosch A, Zimmermann MH. 1983. Evidence for xylem dysfunction by embolization in Dutch elm disease. *Phytopathology* **73**: 1060-1063.

Newman EI. 1976. Water relations. In: Hall MA ed. *Plant Structure and Function*. London: MacMillan Press, 157-196.

Nobel PS, Jordan PW. 1983. Transpiration stream of desert species: resistance and capacitances for a C_3, a C_4, and a CAM plant. *Journal of Experimental Botany* **34**, 1379-1391.

Obuekwe CO, Osagie IJ. 1989. Morphological changes in wilt-resistant and wilt-susceptible oil palm progenies and hydrolytic enzyme activities associated with the *Fusarium oxysporum* f.sp. *Elaeidis* pathogens. *Oleagineux* **44**, 393-401.

Ochs R. Daniel C. 1976. Research on techniques adapted to dry regions. In: Corley RHV, Hardon JJ, Wood BJ, eds. *Developments in Crop Science (1), Oil Palm Research.* Amsterdam: Elsevier Scientific Publishing Company, 315-330.

Oritsejafor JJ. 1989. Status of the oil palm vascular wilt disease in Nigeria. *International Conference on Oil Palm and Palm Products*. Benin City: NIFOR.

Page OT. 1959. Observations on the water economy of *Fusarium*-infected banana plants. *Phytopathology* **49**: 61-65.

Papavizas G. 1967. Evaluation of various media and antimicrobial agents for isolation of *Fusaria* from soil. *Phytopathology* **57**: 848-852.

Park D. 1958. The saprophytic status of *Fusarium oxysporum* Schl. causing vascular wilt of oil palm. *Annals of Botany* **22**:19-35.

Parker RE. 1979. *Introductory Statistics in Biology,* 2nd edition. London: Edward Arnold.

Passioura JB. 1982. Water in the soil-plant-atmosphere continuum. In: Lange OL, Nobel PS, Osmond CB, Ziegler H, eds. *Physiological Plant Ecology II. Water Relations and Carbon Assimilation*. Berlin: Springer-Verlag, 5-35.

Passioura JB. 1988. Response to Dr P. J. Kramer's article, Changing concepts regarding plant water relations, volume 11, number 7, pp. 565-568. *Plant, Cell and Environment* **11**, 569-571.

Passioura JB. 1991. An impasse in plant water relations? *Botanica Acta* **104**: 405-411.

Pearcy RW, Schulze ED, Zimmermann R. 1989. Measurement of transpiration and leaf conductance. In: Pearcy RW, Ehleringer J, Mooney HA, Rundel PW, eds. *Plant Physiological Ecology. Field Methods and Instrumentation*. London: Chapman and Hall, 137-183.

Pegg GF. 1989. Pathogenesis in vascular diseases of plants. In: Tjamos EC, Beckman C, eds. *NATO ASI Series, Series H; Vascular Wilt Diseases of Plants*. Berlin: Springer-Verlag, 51-94.

Pegg GF, Selman IW. 1959. An analysis of the growth response of young tomato plants to infection by *Verticillium albo-atrum*. *Annals of Applied Biology* **47**, 222-231.

Pennypacker BW, Knievel DP, Leath KT, Pell EJ, Hill RR. 1990. Analysis of photosynthesis in resistant and susceptible alfalfa clones infected with *Verticillium albo-atrum*. *Phytopathology* **80**, 1300-1306.

Pennypacker BW, Leath KT. 1986. Anatomical response of a susceptible alfalfa clone infected with *Verticillium albo-atrum*. *Phytopathology* **76**, 522-527.

Phipps PM, Stipes RJ. 1976. Histopathology of mimosa infected with *Fusarium oxysporum* f.sp. *perniciosum*. *Phytopathology* **66**: 839-843.

Ploetz RC. 1990. Population biology of *Fusarium oxysporum* f.sp. *cubense*. In: Ploetz RC, ed. *Fusarium Wilt of Banana*. St Paul: American Phytopathological Society, 63-76.

Porter R. 1989. Development of high yielding oil palm planting material resistant to vascular wilt caused by *Fusarium oxysporum* f.sp. *elaeidis*. *International Conference on Oil Palm and Palm Products*. Benin City: NIFOR.

Potulski N, Barrett JN, Smith BG, Jones L. 1986. *Drought Effect on Oil Palm Ramets, Part 2*. Unilever Research Colworth Laboratory, **Report XCW 86 0104.**

Potulski N, Barrett JN, Smith BG. 1988. *Responses of Irrigated and Droughted Oil Palms to Vapour Pressure Deficit and Temperature*. Unilever Research Colworth Laboratory, Report XCW 88 0030.

Potulski N, Smith BG, Barrett JN. 1988. *Fundamental Oil Palm Responses: Laboratory Experiments January to February 1987*. Unilever Research Colworth Laboratory, **Report** XCW 88 0015.

Prendergast AG. 1957. Observations on the epidemiology of vascular wilt disease of the oil palm (*Elaeis guineensis*, Jacq.). *Journal of the West African Institute for Oil Palm Research* **2**: 148-175.

Prendergast AG. 1963. A method of testing oil palm progenies at the nursery stage for resistance to vascular wilt disease caused by *Fusarium oxysporum* Schl. *Journal of the West African Institute for Oil Palm Research* **4**: 156-175.

Prive JP, Elfving DC, Proctor JTA. 1989. Paclobutrazol, gibberellin and cytokinin effects on growth, development and histology of apple pedicels and fruits. *Journal of the American Society of Horticultural Science* **114**: 273-278.

Purseglove JW. 1985. *Tropical Crops: Monocotyledons*. Harlow: Longman. 479-510.

Rees AR. 1961. Midday closure of stomata in the oil palm, *Elaeis guineensis* Jacq. *Journal of Experimental Botany* **12**: 129.

Reid DM, Wample RL. 1985 Water relations and plant hormones In: *Hormonal Regulation of Development, vol 3* in *Encyclopedia of Plant Physiology, vol 11*. (Ed by R. P. Pharis & D. M. Reid) pp. 513-577. Springer-Verlag, Berlin.

Renard JL. 1967. Incidence de la culture du palmier a huile sur les populations des *Fusarium* dans les sols de savane en Basse Cote-d'Ivore. *Revue de Mycologie* **32**: 211-227.

Renard JL. 1970. La fusariose du palmier a huile. Role des blessures des racines dans le processus d'infection. *Oleagineux* **25**: 581-586.

Renard JL. 1976. Diseases in Africa and South America. In: Corley RHV, Hardon JJ, Wood BJ, eds. *Developments in Crop Science (1), Oil Palm Research*. Amsterdam: Elsevier Scientific Publishing Company, 447-466.

Renard JL, de Franqueville H. 1989. Oil palm vascular wilt. *Oleagineux* **44**: 342-347.

Renard JL, de Franqueville H. 1991. Effectiveness of crop techniques in the integrated control of oil palm vascular wilt. *Oleagineux* **46**: 255-265.

Renard JL, Gascon JP, Bachy A. 1972. Research on vascular wilt disease of the oil palm. *Oleagineux* **27**: 581-591.

Renard JL, Noiret JM, Meunier J. 1980. Sources and ranges of resistance to *Fusarium* wilt in the oil palms *Elaeis guineensis* and *Elaeis melanococca*. *Oleagineux* **35**: 387-393.

Renard JL, Quillec G. 1983. *Fusarium* and replanting. Elements to be considered when replanting oil palm in a *Fusarium* zone in West Africa. *Oleagineux* **38**: 421-427.

Richards RA, Passioura JB. 1989. A breeding program to reduce the diameter of the major xylem vessels in the seminal roots of wheat and its effect on grain yield in rain-fed environments. *Australian Journal of Agricultural Research*. **40**: 943-950.

Robb J, Busch L, Lu BC. 1975. Ultrastructure of wilt syndrome caused by *Verticillium dahliae*. I. In chrysanthemum leaves. *Canadian Journal of Botany*. **53**: 901-913.

Robb J, Smith A, Busch L. 1981. Wilts caused by *Verticillium* species. A cytological survey of vascular alterations in leaves. *Canadian Journal of Botany* **60**, 825-837.

Sackston WE. 1983. Epidemiology and control of seed-borne *Verticillium* spp. causing vascular wilt. *Seed Science and Technology* **11**: 731-747.

Salleo S, Lo Gullo MA, Oliveri F. 1985. Hydraulic parameters measured in 1-year-old twigs of some Mediterranean species with diffuse-porous wood: Changes in hydraulic conductivity and their possible functional significance. *Journal of Experimental Botany* **36**, 1-11.

Salleo S. Lo Gullo MA. 1986. Xylem cavitation in nodes and internodes of whole *Chorisia insignis* H.B. et K. plants subjected to water stress: Relations between xylem conduit size and cavitation. *Annals of Botany* **58**: 431-441.

Sanchez Potez A. 1966. Enfermedades del Algodonero del cocofero y de la palma Africana en Colombia. In : *Review of Applied Mycology* **46**: 630. (abstract).

Scheffer RP, Walker JC. 1953. The physiology of *Fusarium* wilt of tomato. *Phytopathology*. **43**: 116-125.

Scholander PF, Hammel HT, Bradstreet ED, Hemmingsen EA. 1965. Sap pressure in vascular plants. *Science*. **148**: 339-346.

Schulte PJ, Gibson AC, Nobel PS. 1987. Xylem anatomy and hydraulic conductance of *Psilotum nudum*. *American Journal of Botany*. **74**, 1438-1445.

Schulze ED, Gollan ST, Schurr U. 1988. Response to Dr P. J. Kramer's article, Changing concepts regarding plant water relations, volume 11, number 7, pp. 565-568. *Plant, Cell and Environment* **11**, 573-576.

Schulze ED, Hall AE. 1982. Stomatal responses, water loss and CO_2 assimilation rates of plants in contrasting environments. In: Lange OL, Nobel PS, Osmond CB, Ziegler H, eds. *Physiological Plant Ecology II. Water Relations and Carbon Assimilation*. Berlin: Springer-Verlag, 181-230.

Seigel S. 1956. *Nonparametric Statistics for the Behavioral Sciences*. New York: McGraw-Hill Book Company.

Selmann IW, Pegg GW. 1957. An analysis of the growth response of young tomato plants to infection by *Verticillium albo atrum*. *Annals of Applied Biology*. **45**: 674-681.

Shi J, Mueller WC, Beckman CH. 1991. Ultrastructural responses of vessel contact cells in cotton plants resistant or susceptible to infection by *Fusarium oxysporum* f.sp. *vasinfectum*. *Physiological and Molecular Plant Pathology* **38**: 211-222.

Shumway DL, Steiner KC, Abrams MD. 1991. Effects of drought stress on hydraulic architecture of seedlings from five populations of green ash. *Canadian Journal of Botany* **69**: 2158-2164.

Slatyer RO. 1967. *Plant-Water Relationships*. New York: Academic Press.

Smith BG. 1988. *Oil Palm Drought Physiology Analysis of Field Data*. Unilever Research Colworth Laboratory. **Report XCW 88 0335.**

Smith BG. 1989. The effect of soil water and atmospheric vapour pressure deficit on stomatal behaviour and photosynthesis in the oil palm. *Journal of Experimental Botany*. **40**, 647-651.

Smith EF, Gribaudo I, Roberts AV. 1992. Paclobutrazol and reduced humidity improve resistance to wilting of micropropagated grapevine. *Hortscience* **27**: 111-113.

Smith EF, Roberts AV, Mottley J. 1990. The preparation *in vitro* of chrysanthemum for transplantation to soil. *Plant Cell, Tissue and Organ Culture*. **21**: 133-140.

Snyder WC, Smith SN. 1981. Current Status. In: Mace ME, Bell AA, Beckman CH, eds. *Fungal Wilt Diseases of Plants*. New York: Academic Press, 25-50.

Sokal RR, Rohlf FJ. 1981. *Biometry. The Principles and Practices of Statistics in Biological Research*, 2nd edition. London: Freeman and Company.

Sperry JS. 1985. Xylem embolism in the palm *Rhapis excelsa*. *IAWA Bulletin*. **6**: 283-292.

Sperry JS. 1986. Relationship of xylem embolism to xylem pressure potential, stomatal closure, and shoot morphology in the palm *Rhapis excelsa*. *Plant Physiology* **80**: 110-116.

Sperry JS, Donnelly JR, Tyree MT. 1988. A method for measuring hydraulic conductivity and embolism in xylem. *Plant Cell and Environment* **11**: 35-40.

Sperry JS, Perry AH, Sullivan JEM. 1991. Pit membrane degradation and air-embolism formation in ageing xylem vessels of *Populus tremuloides* Michx. *Journal of Experimental Botany* **42**: 1399-1406.

Sperry JS, Tyree MT. 1988. Mechanism of water stress-induced xylem embolism. *Plant Physiology* **88**: 581-587.

Sperry JS, Tyree MT. 1990. Water-stress-induced xylem embolism in three species of conifers. *Plant Cell and Environment* **13**: 427-436.

Stathers T. *Investigation of Fusarium Oxysporum f.sp. elaeidis on Oil Palm with Particular Reference to Decontamination of Pollen and Pathogenicity of Nitrate Non-utilizing Mutants. Report for Honours Degree in Applied Biology*. University of Bath.

Street PFS, Cooper RM. 1984. Quantitative measurement of vascular flow in petioles of healthy and *Verticillium*-infected tomato. *Plant Pathology*. **33**: 483-492.

Street PFS, Robb J, Ellis BE. 1986. Secretions of vascular coating compounds by xylem parenchyma cells of tomatoes infected with *Verticillium albo-atrum*. *Protoplasma* **132**: 1-11.

Stuckmeyer BE, Beckman CH, Kuntz JE, Riker AJ. 1954. Plugging of vessels by tyloses and gums in wilting oaks. *Phytopathology* **44**: 148-152.

Talboys PW. 1968. Water deficits in vascular disease. In: Kozlowski TT, ed. *Water deficits and Plant Growth*. New York: Academic Press, 255-311.

Taquet B. 1985. *Les Mechanismes Physiologiques de la Reaction de Defence du Palmier a Huile (Elaeis guinneensis Jacq) Contre la Fusariose Vasculaire. Application a la Recherche de Nouveaux Moyens de Lutte*. PhD Thesis, University of Pierre et Marie Curie, Paris.

Terashima I, Wong S, Osmond CB, Farquhar GD. 1988. Characterisation of non-uniform photosynthesis induced by abscisic acid in leaves having different mesophyll anatomies. *Plant Cell Physiology* **29**, 385-394.

Threlfall RJ. 1959. Physiological studies on the *Verticillium* wilt disease of tomato. *Annals of Applied Biology*. **47**: 57-77.

Tomlinson PB. 1990. *The Structural Biology of Palms*. Oxford: Clarendon Press.

Trique B. 1971. Pectinases et acide fusarique du *Fusarium oxysporum* f.sp. *elaeidis*, leurs roles dans la fusariose du palmier a huile. *Oleagineux* **26**: 163-168.

Turner P. 1981. *Oil Palm Diseases and Disorders*. Oxford: Oxford University Press.

Turner SL. 1992. *Studies of Fusarium Wilt of Oil Palm with Reference to Seed Decontamination and Soil Epidemiology*. Report for Honours Degree in Applied Biology. University of Bath.

Tyree MT, Dixon MA, Tyree EL, Johnson R. 1984. Ultrasonic acoustic emission from the sapwood of cedar and hemlock. *Plant Physiology*. **75**: 988-992.

Tyree MT, Ewers FW. 1991. Tansley review no 34. The hydraulic architecture of trees and other woody plants. *New Phytologist* **119**, 345-360.

Tyree MT, Hammel HT. 1972. The measurement of the turgor pressure and the water relations of plants by the pressure-bomb technique. *Journal of Experimental Botany*. **23**: 267-282.

Tyree MT, Sperry JS. 1988. Do woody plants operate near the point of catastrophic xylem dysfunction caused by dynamic water stress? *Plant Physiology* **88**: 574-580.

Tyree MT, Yang S. 1990. Water storage capacity of *Thuja*, *Tsuga* and *Acer* stems measured by dehydration isotherms: contributions of capillary water and cavitation. *Planta* **182**, 420-426.

Tzeng DD, Wakeman RJ, DeVay JE. 1985. Relationships among *Verticillium* wilt development, leaf water potential, phenology, and lint yield in cotton. *Physiological Plant Pathology* **26**: 73-81.

Van Alfen NK. 1989. Reassessment of plant wilt toxins. *Annual Review of Phytopathology* **27**, 533-550.

Van Alfen NK, Allard-Turner V. 1979. Susceptibility of plants to vascular disruption by macromolecules. *Plant Physiology* **63**: 1072-1075.

Van Alfen NK, McMillan BD, Turner V, Hess WM. 1983. Role of pit membranes in macro molecule-induced wilt of plants. *Plant Physiology* **73**: 1020-1023.

Van Alfen NK, McMillan BD, Wang Y. 1987. Properties of the extracellular polysaccharides of *Clavibacter michiganense* subsp. *insidiosum* that may affect pathogenesis. *Phytopathology* **77**: 501-505.

Van Alfen NK, Turner NC. 1975. Changes in alfalfa stem conductance induced by *Corynebacterium insidiosum*. *Plant Physiology* **45**: 304-309.

Van de Lande HL. 1983. Vascular wilt disease of oil palm (*Elaeis guineensis*) in Para, Brazil. *Oil Palm News* **28**: 6-10.

Vanderplank JE. 1982. *Host-Pathogen Interactions in Plant Disease*. New York: Academic Press.

Vanderplank JE. 1984. *Disease Resistance in Plants*. Second edition. New York: Academic Press.

Veres JS. 1990. Xylem anatomy and hydraulic conductance of Costa Rican *Blechnum* ferns. *American Journal of Botany* **77**, 1610-1625.

Villalobos E, Umana UH, Chinchilla C. 1992. Oil palm water status in response to drought in Costa Rica. *Oleagineux* **47**: 1-7.

Wang SY, Steffens GL. 1985. Effect of paclobutrazol on water stress-induced ethylene biosynthesis and polyamine accumulation in apple seedling leaves. *Phytochemistry* **24**: 2185-2190.

Wang SY, Sun T, Zuo L, Faust M. 1987. Effect of paclobutrazol on water stress-induced abscisic acid in apple seedling leaves. *Plant Physiology* **84**: 1051-1054.

Wardlaw CW 1946a. A wilt disease of the oil palm. *Nature* **158**: 56.

Wardlaw CW. 1946b. *Fusarium oxysporum* on the oil palm. *Nature* **158**: 712.

Wardlaw CW. 1946c. *Observations on Diseases of the Oil Palm in the Belgian Congo*. Unilever Internal Report.

Wardlaw CW. 1948. Vascular wilt disease of oil palm in Nigeria. *Nature* **162**: 850-851.

Wardlaw CW. 1950. Vascular wilt disease of the oil palm caused by *Fusarium oxysporum* Schl. *Tropical Agriculture* **27**: 42-47.

Waterson JM. 1953. Observations on the influence of some ecological factors on the incidence of oil palm diseases in Nigeria. *Journal of the West African Institute for Oil Palm Research* **1**: 24-59.

West J. 1944. Oil palm diseases in Nigeria. *Internal Memo, Department of Agriculture Nigeria*.

Wieland WF, Wample RL. 1985. Root growth, water relations and mineral uptake of young "delicious" apple trees treated with soil and stem applied paclobutrazol. *Scientia Horticulturae* **26**: 129 137.

Worthing CR, Walker SB. 1983. *The Pesticide Manual*. Seventh edition. London: British Crop Protection Council.

Wymore LA, Baker R. 1982. Factors affecting cross-protection in control of Fusarium wilt of tomato. *Plant Disease* **66**: 908-910.

Zimmermann MH. 1983. *Xylem Structure and the Ascent of Sap*. Berlin: Springer-Verlag.

Zimmermann MH, Brown CL. 1971. *Trees Structure and Function*. Berlin: Springer-Verlag.

Zimmermann MH, McDonough. 1978. Dysfunction in the flow of water. In: Horsfal J, Cowly E, eds. *Plant Disease, an Advanced Treatise Vol III*. New York: Academic Press, 117-140.

Zimmermann MH, Sperry JS. 1983. Anatomy of the palm *Rhapis excelsa*. IX. Xylem structure of the leaf insertion. *Journal of the Arnold Arboretum* **64**: 599-609.

APPENDICES

Appendix 1

Preparation of Media

All media were sterilized at 121°C for 15 minutes.

1. Sucrose (*Fusarium*) Salts Medium (FSM)
(Cooper & Wood, 1975)

	g l^{-1}
$NaNO_3$	2
KH_2PO_4	1
$MgSO_4.7H_2O$	0.5
Sucrose	15

Trace element stock solution 10ml l^{-1}

Trace element stock solution:-

	mg l^{-1}
$FeSo_4.7H_2O$	20
$ZnSO_4.7h_2O$	100
$NaMoO_4.2H_2O$	2
$CuSo_4.5H_2O$	2
$MnCl_4.H_2O$	2

The medium was made up to 1 Litre in distilled water and adjusted to pH 6.5 before sterilization.

2. Papavizas Medium (*Fusarium* Selective Medium)
(Papavizas, 1967)

	g l^{-1}
KH_2PO_4	1
Peptone	5
$MgSO_4.7H_2O$	0.5
Botrilex (20% a.i. Pentachloranitrobenzene)	5
Agar	12

The medium was made up to 1 Litre in distilled water. The following antimicrobial agents were added to the medium as it cooled after sterilization:- 0.05g Chloramphenicol, 0.3g Penicillin, 0.134g Streptomycin Sulphate.

3. Potato Dextrose Agar (PDA)

39g of PDA powder (London Analytical & Bacteriological Media Ltd, London) was added to 1 Litre of distilled water.

4. Pollen Germination Agar
(Brewbaker & Kwack, 1963)

	$g\ l^{-1}$
Agar	20
Sucrose	40
H_3Bo_3	0.1
$Ca(NO_3)_2.4H_2O$	0.3
$MgSO_4.7H_2O$	0.2
KNO_3	0.1

The medium was made up to 1 Litre in distilled water and adjusted to pH 6.5 before sterilization.

Appendix 2

Calculation of Transpiration from Stomatal and Boundary Layer Conductances (from Nobel & Jordan, 1983)

Transpiration (Mol m^{-2} s^{-1}) = leaf conductance (Mol m^{-2} s^{-1}) x water vapour gradient (mol mol^{-1}).

A. Calculation of Leaf Conductance.

$$\frac{1}{\text{Leaf Conductance}} = \frac{1}{\text{Stomatal Conductance}} + \frac{1}{\text{Boundary Layer Conductance}}$$

Stomatal conductance (Mol m^{-2} s^{-1}) was obtained from porometer measurements of abaxial stomatal resistance (2.3.4.b.) converted to conductance (mm s^{-1}) then to Mol m^{-2} s^{-1} (Table A9, Pearcy *et al*, 1989). Values of adaxial conductance were very low and were therefore usually ignored. But in experiments with severely water stressed

leaves stomatal conductance included an estimate of adaxial conductance (0.75 mMol m^{-2} s^{-1}, based on the lowest abaxial conductance value).

Boundary layer conductance ($m\ s^{-1}$) = $\frac{\text{diffusion coefficient}(m2\ s\text{-}1)}{\text{boundary layer thickness (m)}}$

(Nobel, 1983.). Values were converted to Mol m^{-2} s^{-1} as for stomatal conductance above.

Boundary layer thickness (mm) = $4 \sqrt{L \div V}$ (Nobel, 1983)

L = mean length of leaf (m)

V = ambient air speed ($m\ s^{-1}$)

Air speed was measured with a TA6000 hot wire anemometer (Air Flow Laboratories, High Wycombe)

B. Calculation of Vapour Pressure Gradient

The gradient is the difference in mole fraction of water vapour inside the leaf and ambient air (Pearcy *et al*, 1989).

Inside leaf mole fraction = saturated vapour pressure at leaf temperature (KPa) divided by total air pressure (KPa).

Ambient air mole fraction = vapour pressure at ambient air temperature and humidity (KPa) divided by total air pressure (KPa).

For estimation of hydraulic resistance transpiration values were converted to units of m^3 m^{-2} s^{-1}. Leaf temperature was measured by the porometer and air temperature and humidity by a recently calibrated

thermohydrograph (Cassella,London), which was kept in a box through which air was conducted by a fan.

Appendix 3

Mass production of Inoculum of *Fusarium oxysporum*.

A 3 litre flask containing 1.3 litres of sterile sucrose salts media (appendix 1) was inoculated with 140 ml of a spore suspension of 2.5 x 10^7 spores/ml. The flask was aerated by use of a pump blowing air through a sterile filter to an aquarium air stone in the media. After 3 days incubation at 27^{o}C the flask yielded 1.3 litres of a spore suspension of 2 x 10^8 spores/ml. This would be enough to inoculate almost 800 plants with 10 ml of 3.3 x 10^7 spores/ml.

Appendix 4

Suppliers of Fungicides.

Benomyl, Du Pont (UK) Ltd, Stevenage.
Captafol, ICI Agrochemicals, Farnham.
Chlorothalonil, ICI Agrochemicals, Farnham.
Prochloraz, Schering Agriculture, Nottingham.
Imazalil, Hortichem, Salisbury.
Iprodione, Hortichem, Salisbury.

Appendix 5

Isolates of Foe were imported and retained under licence (PHF 343/134(107)) issued by the Ministry of Agriculture Fisheries and Food.